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Fifth International Congress on Nutrition

The Fifth International Congress on Nutrition, held in Washington, D. C., September 1–7, 1960, opened in Plenary Session with an address by Dwight D. Eisenhower, President of the United States. Some 1800 scientists, representing 69 countries, participated in the week of discussions and reports on current nutrition research.

The scientific sessions of the Congress implemented an important exchange of knowledge on the major problems of nutrition throughout the world. One of the basic contributions of the Congress was the evaluation of the status of nutrition information and the identification of areas where the need for research is urgent. Topics covered the spectrum of nutrition from undernutrition to overnutrition. The scientific program consisted of an all-day symposium on "World Food Needs and Food Resources"; seven sessions of panel discussions by invited speakers; and 367 contributed papers on original, unpublished research presented in concurrent sessions. Simultaneous translation into English, Spanish, French, and German was provided. Scientific sessions were supplemented by industrial and institutional exhibits.

Several agencies of the United States Government, foundations, institutions, and industry provided financial support for the Congress.

An International Congress on Nutrition is held every three years under the auspices of the International Union of Nutritional Sciences. The International Union, organized at the First Congress in London in 1946, has demonstrated its effectiveness in accomplishing efficient communication among nutritional scientists from all parts of the world, increasing the number of exchange fellowships, promoting high standards of nutrition research, and maintaining effective liaison with other international bodies interested primarily in nutrition advancement.

Previous Congresses have been held in Switzerland, The Netherlands, and France. The Fifth Congress was the first to be held in the Western Hemisphere. The Sixth Congress will convene in 1963 in Edinburgh, Scotland.

Fifth International Congress on Nutrition

Opening Plenary Session

Welcome

C. GLEN KING, President of the Congress

ON BEHALF OF OUR HOST ORGANIZATIONS, I have the honor and great pleasure of welcoming you to this meeting of the International Union of Nutritional Sciences in Washington.

The American Institute of Nutrition has shared with the National Academy of Sciences–National Research Council and with many other organizations during the past four years in making plans for our sessions here. We are indebted also to the International officers, Dr. David O. Cuthbertson, Chairman, and Dr. Leslie Harris, Secretary-General, for their excellent cooperation.

Especially do I want to acknowledge our indebtedness for the persistent hard work by the organizing committee under the chairmanship of Dr. Paul György, and by the many special committees and subcommittees who met their responsibilities so well. I want particularly to express our gratitude to Mr. H. J. Heinz II whose services as honorary chairman of the Finance Committee were a major factor in our obtaining substantial support from industry. Special acknowledgment is due also to the Executive Committee Members—to Dr. W. Henry Sebrell, Jr., Chairman of the Program Committee, to Dr. Floyd S. Daft, Chairman of the Hospitality Committee, and to his assistants for the Women's Program, Mrs. C. Glen King and Mrs. E. M. Nelson. Likewise, the members of the staff associated with the General Secretary, Dr. Milton Lee, have met their administrative tasks with a friendly spirit and notable performance. We thank especially Mrs. Helena B. Lemp and Miss Sara F. Leslie who served respectively as Convention Manager and Executive Editor.

Most cheerfully, we acknowledge our gratitude to those who have come to share in the program, including those who speak and those who listen, because we have confidence that everyone here will face into the future better prepared to do important work for the betterment of mankind.

We are now privileged to receive greetings from: Mr. Maurice Pate, Executive Director of the United Nations Children's Fund, representing the United Nations; Dr. Vincent du Vigneaud, the National Academy of Sciences; Dr. Floyd Daft, the American Institute of Nutrition; Dr. David Cuthbertson, the International Union of Nutritional Sciences; Dr. E. W. McHenry, the Nutrition Society of Canada; and Dr. Abraham Horwitz, the Pan American Health Organization.

We could have no greater honor or pleasure, than to be addressed by our next speaker. Ladies and Gentlemen:

The President of the United States of America!

Address

DWIGHT D. EISENHOWER, President of the United States of America

Dr. King, and Ladies and Gentlemen:

I am delighted to open this Fifth International Congress on Nutrition, a Congress attended by representatives from fifty-nine countries, including every continent on the globe. Since you have previously met in London, Basle, Amsterdam, and Paris, but this is your first visit to Washington, I bid you a hearty welcome to this side of the Atlantic and to this City.

May I remark, President King, that I envy you in your association with this Congress. You will not need to veto any of its actions. Now this, of course, is because each of you is selflessly and wholeheartedly dedicated to the advancement of a science that underlies human health. You have come with a vision to build a better world, now and for years to come.

The Twentieth Century is unique in many ways—not the least of which is the fact that ours is the first generation which has dared to think in terms of food enough for all. And our age is the first to be deeply concerned about the quality as well as the quantity of the food supply. For the first time in history, man's ancient enemies—hunger and malnutrition—are on the defensive. They are not whipped. But ours is the first generation to catch the scent of victory.

Let me turn for a moment to one phase of the free world's campaign against hunger, a program to send crop surpluses to needy areas. I take as an example the case with which I am most familiar, that of my own country. But first a word of caution. Any transaction involving the transfer of commodities from one nation to another is of more than bi-lateral interest. Thus, in moving our abundant surplus of food products overseas, we must be diligent to avoid disrupting the markets of others. Irresponsible handling of our huge stocks of wheat, for example, could unjustifiably harm a nation which is heavily dependent on foreign exchange earnings from wheat and other cereal exports. My concern regarding this problem is one of the reasons for recommending increased use of the United Nations so as to distribute surplus crops under methods that will benefit all.

During the past six years, the United States government has sent more than four thousand shiploads of food abroad in exchange for foreign currencies.

In similar transactions, we have done or engaged to do things like the following—to one country 16 million tons of wheat and one million of rice; for disaster relief, in earthquakes and hurricanes, 300 shiploads of food have gone abroad—through voluntary charitable agencies 400 shiploads of food to help 60 million stricken people.

Twelve hundred United States agricultural technicians are now working overseas, translating agricultural science into better living for the world's millions. Last year we received more than three thousand agricultural visitors from other countries, who came here to study food production, agricultural research and education, and to meet our farmers and to see how they work.

We have loaned over $265 million abroad to build irrigation projects, fertilizer plants, and to improve transportation facilities.

Now these activities of my own country are only a part of the total free world program to lift the scourge of hunger. Great efforts are being made by the developing countries themselves. Much help has come from other industrialized nations. The special agencies of the United Nations—the World Health Organization, the United Nations Children's Fund and the Food and Agriculture Organization—have all made outstanding contributions in our common effort to eliminate hunger from this planet.

And the combined effort has been effective. There have been no major famines in the Free World during the past decade and, to my knowledge, this cannot be said of any previous decade. Nutritional levels in most of the developing countries, while still distressingly low, have nevertheless crept up slightly. World agriculture has generally kept abreast or ahead of the population increase.

While we have thus helped lift production capabilities abroad, the stream of agricultural and industrial exports from the more industrialized nations has increased, not diminished. The reason is a simple one: a better-fed neighbor is a better customer.

This is as it should be, and reflects the wisdom of programs which meet current needs while building long-term self-reliance. To make the recipient countries indefinitely dependent upon our assistance would be disadvantageous to them and to us. Compassion and prudence are equally important in this undertaking; our Food-for-Peace program partakes of both.

There is a Danish proverb which says: "You may light another's candle at your own without loss." Indeed there is gain in the lighting of many candles; in the brighter light we can all see better.

There are risks, indeed, in our undertaking. But the risks of failing to face up to our opportunities are greater

than those involved in considered action. Political explosions can result, in a shrinking world, from a widening gap between the wealthy and the underdeveloped nations.

And science has given us a set of tools designed for human betterment. Farm people, in the United States and elsewhere, have translated these tools into a capability for constructive action. Though the task is gigantic, we seek opportunity to move ahead rather than becoming preoccupied with despair.

The world cups its ear to hear the rattling of rockets. It listens less closely to the sounds of peace and well-being which emanate from the slow but steady improvement in world health and nutrition.

For centuries orators and writers have developed the habit of warning about the crossroads that the world was facing at the very moment of the particular speaking or writing. Many of these crossroads have existed only in a lively imagination. Yet if history, which will one day view the events of this period in perspective, could only say that it was at this moment the world began truly to take the high road of health, and plenty, leading toward peace, leaving forever the path of strife and anxiety, then indeed would our great grandchildren call this the brightest era of all time.

To each of you, my best wishes for a successful Congress. To the degree that you succeed, the human family in the nations here represented will step from under the shadow of want. This is the purpose that has brought you half-way around the world. The earth's nearly three billion people join me, I am sure, in my good wishes for your success. And may God ever be your Helper.

Thank you very much.

PANEL I

Evaluation of Nutritional Status in Man

Chairman: Grace A. Goldsmith, USA
Moderator: E. W. McHenry, Canada

Interpretation of nutritional requirements in terms of action programs

E. W. McHENRY
School of Hygiene, University of Toronto, Canada

THIS PANEL DISCUSSION is concerned with the reliability of methods of appraising nutrition conditions in a community and the interpretation of data arising from such a study. Originally, I agreed to act as moderator of the panel. You can appreciate my shocked surprise when I learned, indirectly, that I was supposed to contribute a formal presentation on a subject about which I know very little. In fact, I do not even know the meaning of the assigned title. It might be wise to consider first, possible meanings of the title.

The term, 'nutritional requirements' can be very misleading. Do we mean minimal or optimal needs? To many people nutritional requirements mean that a housewife, aged 25 and in normal health, requires 2400 Calories a day; if she gets only 2300, she is undernourished. Very few people appreciate that this housewife will be overnourished if she has an intake of 2500 Calories a day for an extended period, at the end of which she will be overweight and will need to shift to Metracal for weeks or months. If she is living in an area in which food is scarce, she may be fortunate to have 2000 Calories a day; if so, she will be underprivileged and needing food from the surplus stocks in the United States or Canada. A physical examination would probably show that she is a bit underweight, but otherwise healthy.

In planning feeding programs, a well-trained nutritionist will decide that every housewife must have sufficient food to provide 2400 Calories a day. I think this is one possible meaning of the interesting phrase, 'action programs,' in the title assigned to me.

In many areas in Asia and Africa sufficient food to provide a housewife with 2400 Calories a day will not be available and the importation of foods will be necessary. Payment for the imported foods may present a very real problem. Some, at least, of the imported foods may be strange to our housewife and an educational program to get her to eat these new foods will be essential. There arises, at once, the question about the personnel to carry out the educational program. An appeal will be made to FAO or WHO or another international agency to supply a nutritionist for work in the community. The appeal may or may not be successful. Some time in the future the person responsible for the original recommendation will learn that people can and do adapt themselves to intakes less than recommended allowances. At first, she will be shocked to find out that the housewife is surviving on a protein intake of about 45 grams a day, of which very little is animal-source protein. If the housewife becomes pregnant and carries on, presently giving birth to a small, but reasonably healthy baby, the shock will change to credulous disbelief. Obviously, there was something wrong with the nutrition training of the nutritionist. One of the major difficulties is the tendency of people, particularly educated ones, to believe that everything which appears in print is true. I feel that this is a serious problem with the interpretation of recommended allowances and of other statements of nutritional requirements, especially in terms of action programs. Very few nutritionists realize that our knowledge of nutritional requirements is far from precise and is, indeed, sketchy. The foolishness of expressing requirements in terms of plus or minus 10% is seldom appreciated. Sufficient information may be available to express the thiamine need of a 100-gram rat within the limits of 90 to 110% but comparable information is not available for humans of any age or size.

The lack of information will not prevent students in nutrition from being taught and, hence, memorizing that our housewife should have 2400 Calories, 70 grams of protein and 1.2 mg thiamine per day. I suggest that the teaching should be that the housewife needs about 2400 Calories, about 70 grams of protein and about 1 mg thiamine a day and that she may be adapted to intakes of 2000 Calories, 45 grams of protein and 0.8 mg thiamine a day. I hope Miss Copping won't mind if I point out that, if this information has been obtained from a study of food intake, there are probably people rushing around looking for nonexistent beri beri. In 1944, H. H. Mitchell described the existence and the practical importance of nutrition adaptation; the description has been largely ignored. We know now that nutrition adaptation does occur in many areas of the world, that pregnancy is a time when nutrition adaptation with regard to protein, calcium, iron and other nutrients is clearly evident, and that there is information available regarding the mechanisms of adaptation. This information makes advisable critical appraisal of our so-called knowledge of nutritional requirements

and of results of studies of food intake. The customary procedure of doing a study of food use and then stating that a given percentage of the subjects were, or are, undernourished, should be discontinued. The planning of action programs on doubtful interpretation of data of dubious value will no longer be satisfactory. I suspect that we would all benefit from a slow and careful rereading of Bulletin 109 of the U.S. National Research Council. This bulletin, published in 1943, has the interesting title: "Inadequate Diets and Nutritional Deficiencies in the U.S." From data on food consumption it was calculated that a considerable number of Americans were deficient in thiamine, riboflavin and nicotinic acid. This conclusion became the principal argument for the flour enrichment program.

I hope that it is now clear that I am suggesting that published statements of nutritional requirements should be altered to be in accord with our knowledge or ignorance. If this were done, it is likely that nutrition teaching would be changed and that interpretation of nutritional requirements would alter markedly. Such a change would have a real affect on 'action programs.'

Sampling, organization, and general plan for the evaluation of nutritional status in man

ARNOLD E. SCHAEFER

Interdepartmental Committee on Nutrition for National Defense, National Institutes of Health, Bethesda, Maryland, USA

THE NUTRITIONAL STATUS of a population is influenced by many factors, such as geography, agriculture, transportation, education, economics, social mores, and infectious and parasitic diseases. Manifestations of undernutrition and malnutrition vary considerably in degree and type in different countries, and in most cases in different areas within a country. Estimates, or even fairly accurate data, on average caloric and nutrient intake are not sufficient for accurate evaluation of nutritional status since inequities in distribution, availability of nutrients in food, and many other factors may influence the situation as far as the individual is concerned. The consumption of a diet that is marginal in protective nutrients may permit survival for an indefinite period. However, populations subsisting at this nutritional level usually have a low life expectancy, increased disease rates, lowered physical and mental efficiency, and other manifestations of ill health.

One is concerned in nutrition surveys with the problem of assessing nutritional levels within the population, their variation, the factors which influence these nutritional levels and the opportunities for their betterment. Nutritional appraisals of this type should not be confused with the earlier studies to 'detect and highlight' occult nutritional deficiency diseases. The term 'nutrition survey' has a multiplicity of meanings. To some it connotes an epidemiological survey of specific nutritional diseases, or of selected population groups such as school children, or a combination of these. Similarly, the methods employed in nutrition surveys may be limited to one assessment method or a combination of physical, biochemical, dietary, and agricultural appraisals.

This discussion shall consider the procedures given in the "Manual for Nutrition Surveys" (6) which were developed by the Interdepartmental Committee on Nutrition for National Defense (ICNND) as a guideline for assessing the nutritional status of the population of a country. The principal purpose of this manual was to establish uniformity in methods, techniques, and procedures, so that a reliable comparison could be made of the findings within and among countries. Based on experience gained in nutrition surveys conducted to date in 14 countries by the ICNND, it is apparent that a combined approach including physical, biochemical, and dietary assessment gives more meaningful information than any one approach taken separately. For evaluation of the capabilities for nutrition improvement it is essential that the dietary data be supported with a food and agriculture appraisal, including the type and amounts of food consumed, food supply, production, processing, distribution, and economics. In addition, supplementary health data on such factors as sanitation and the incidence of parasitic and infectious diseases should be evaluated as to their possible impact on nutritional health.

Limitations of individual methods of appraisal. Physical examinations can in general be extended to a much larger population sample than biochemical and dietary appraisals. However, there are a number of problems inherent in the interpretation of the data. Experience demonstrates that there is less likelihood of divergence of opinion on standards by examiners in the instance of frank deficiency diseases, but that those characteristics which are dependent upon slight differences usually noted in suboptimal cases of nutritional deficiency are susceptible to pronounced differences of opinion among physicians. Few if any of the physical signs of suboptimal nutrition in mild forms are specifically diagnosed. Furthermore, it is known that physical signs of nutrient deficiencies come and go, often unpredictably, in mild deficiency states. In general, the physical signs, in conjunction with the biochemical findings, represent the results of dietary habits of long duration.

Some of the biochemical values, such as blood serum or urinary levels of vitamin C and some of the B-complex vitamins can also be misleading in that they can be influenced by fairly recent dietary changes. Biochemical findings are extremely valuable in assessing suboptimal nutrition where physical symptomology cannot be recognized. For example, at the time of the physical examination, some of the nutrient levels of the blood and urine may be extremely low, with the subject showing no physical signs of these deficiencies.

Dietary studies constitute an essential part of any complete nutrition survey. Populations which, for extensive periods, subsist on dietary intakes which are

marginal in protective nutrients may fail to reveal any recognized specific nutritional deficiency lesions. Under such conditions, where clinical observations are insensitive for detecting nutritional problems, nutrient intake studies are essential. Dietary studies not only yield information on the adequacy of existing diets, but also involve collection of information concerning food habits, ration allowances, menu preparation, food procurement and distribution, food waste, and nutritional adequacy of foods available and of those actually consumed. Although both clinical examinations and biochemical tests provide direct means of studying the nutritional status of individuals, the basic information collected in dietary studies is necessary to plan the changes in nutritional practices that may be required. In addition, the same information is needed when it is desired to modify existing economic, agricultural and food management policy and programs to assure the best use of available food supplies. It must be appreciated that dietary data represent only the situation at a given limited time. Seasonal foods and other variations of dietary intake might alter significantly the nutritional status of the individual.

The nutrition survey is likely to uncover more problems than it solves. To use the data and findings intelligently and effectively requires additional follow-up work, often further research, confirmation of the findings by such procedures as therapeutic trials, saturation tests, and the use of additional diagnostic tests which may be more specific. These may have to be carried out on a smaller sample of the population. However, due to their complexity such studies are often not adaptable for use in field surveys of a large number of samples.

PLAN AND ORGANIZATION

Initial arrangements. Due to the fact that many disciplines are essential for improving nutrition and health, it is vital that the early negotiations with the government concerned be carried on at a 'high level.' The governmental agencies concerned with Health, Agriculture, Development, Education, and Defense should be adequately briefed as to the purpose and objectives of the study. The success of the mission depends upon the assistance and cooperation of all these agencies. Their interest and cooperation are especially important in implementing the possible recommendations for nutritional improvement. It is indeed helpful to have a preliminary general discussion period with representatives of these agencies attending. Members of other cooperating agencies in the country, such as U.N. agencies and representatives of bilateral countries, missions, or programs should also be included. In general, a nutrition survey contributes greatly to a closer integration and understanding among the cooperating agencies. The survey by its very nature, purpose and objectives cuts across departmental lines. Upon receiving a request for a survey from the government concerned, preparation of a detailed plan is necessary. For this purpose it is indeed helpful to have the governmental and cooperating agencies appoint a technical working group. This group should include individuals familiar with the geography and population of the country, food habits, customs, and food production, and a specialist in transportation logistics.

Arrangements should be made for ancillary studies to be conducted in conjunction with the survey. These should include parasitological studies. There are often scientists in other laboratories or institutes who will cooperate in studies such as: serum electrophoresis; determination of serum cholesterol and fatty acids; blood typing; electrocardiogram studies; and trace element or mineral determinations in blood and urine. The extent of the ancillary studies should, however, not impair the primary mission of the survey.

Background. Just as a physician records the history of his patient upon the initial visit, it is essential for a background report to be prepared for the team members to assist in the preparation of the survey plans. This background of the history of the country or area should include general information regarding geography, transportation, communication, population statistics, birth and death rates, principal causes of death, location of hospitals, research centers, and missions, the degree of literacy, languages spoken, and social customs and mores that affect the dietary pattern of the population. This report should also contain a fairly detailed appraisal of the quantities of foods produced, imports, exports, food processing, storage and distribution, and laws and regulations regarding food standards. It should also include, whenever possible, the economic and work classification of the various population subgroups. This background report, based primarily on existing reports and the available data, should be checked and verified during the survey.

Selection of areas to be surveyed. In this discussion I am assuming that the initial appraisal of the nutritional status of the population in question will be accomplished in a minimum period of time, a period of approximately 60 to 90 working days in the field. The first selection should be based on the major areas in which one would anticipate variations in nutritional status. The process of selection of general areas to be surveyed usually begins with a definition or outline of those areas of the population known to have different food habits which in essence are usually based upon the type of agricultural products grown in those regions. The areas may then be further subdivided according to differences in racial, social, and economic considerations. In the initial planning, subgroups or areas should be assigned priorities. Thus, it may be that 30 subareas are initially selected and during the time allotted for the survey only 20 can be visited. Further subgroups or areas for sampling can be established when deemed desirable, such as rural vs. urban, or types of occupation. Sampling in the various areas will include the usual family breakdown; viz., males, females, infants, pregnant women, etc.

Team composition. The size of the team obviously is

dependent upon the magnitude of the projected survey. For this discussion I should like to refer to the usual composition of the nutrition teams operating under the auspices of the ICNND, keeping in mind that these surveys have had a dual mission, not only to assess nutritional status, but to assist in training host country personnel in the techniques of nutritional appraisal. The survey team must have a director whose primary mission is to insure integration of the various methods employed. It is only fair to point out that this individual more frequently than not finds himself involved in the frustration of logistics and protocol. To survey countries such as Ethiopia, Ecuador, Chile, and Vietnam, the following team composition has worked quite satisfactorily:

1) Survey director—1, and 1 administrative or transportation officer
2) Base laboratory group—3 to 6 biochemists, 2 laboratory aides, one secretary
3) Clinical group—2 to 6 physicians (trained in clinical nutrition), 2 to 4 technicians, 1 or 2 dentists and 1 or 2 assistants
4) Dietary survey group—2 to 4 nutritionists (trained in dietary survey techniques and food handling and distribution), 2 to 4 dietitians or similar trained individuals to assist in home or questionnaire studies
5) Food and agricultural specialist—1 or 2
6) Statistician—1

Team briefing. It is desirable to assemble the team members approximately 1 to 2 months prior to the survey for a general briefing. This enables the group to discuss the background report, the survey plans, become familiar with the procedures to be employed and to recognize that each group and individual has a distinct contribution to make to the over-all nutritional appraisal. Once the survey is in operation, routinely scheduled briefing sessions should be held to discuss the impressions and findings of the various team members.

Logistic support. The headquarters or base of operations is usually located at the main base laboratory. Selection of the laboratory site should, above all, consider the future possibility and potential for continued utilization in further nutrition studies, food analysis, and clinical and biochemical research. The laboratory equipment and supplies needed are similar to the requirements for the usual clinical-biochemistry laboratories. A complete list of equipment and supplies has been published (6). In general, the laboratory supplies and equipment, to continue to function considerably beyond the nutrition survey, cost approximately $10,000 to $12,000. Expanding the laboratory to include food analysis requires an additional $5,000 to $8,000. The actual housing for the laboratory requires approximately 500 square feet.

Arrangements for transportation of the team to the field require meticulous planning and agreement. A word of caution is that this can never be worked out in too great detail. Having the Armed Forces or a similar organization of the country cooperate in the surveys is indeed of tremendous advantage in providing transportation within the country. The procedure used for the ICNND surveys has been to ship blood, urine, food, and parasitologic specimens back to the base laboratory by air. The team members, depending upon distances, have flown to major area locations. Ground transportation is then utilized throughout that area. For a field team of 15 to 20 members, ground transport requirement is approximately 5 jeeps or similar vehicles and 1 truck. In addition, 1 vehicle is needed at the base laboratory.

PLAN OF OPERATION

Advance party. It is desirable to have an advance group organize the initial phases of the survey prior to assembling the entire team. This group should include at least the following: team director, 1 clinician, administrative officer, 1 nutritionist, statistician, and the key laboratory personnel. The major tasks include protocol visits, preparation of the survey itinerary and issuance of detailed travel plan and orders.

The laboratory personnel have the responsibility to check the equipment and supplies and to familiarize themselves with and standardize the biochemical methods. The senior physician has the responsibility to orient and train his group and to standardize the physical diagnostic criteria among them.

Initially the principal indicator lesions of nutritional deficiency can be studied by the use of colored photographs. For further indoctrination 3 to 5 subjects obtained from hospitals or outpatient clinics are given a complete examination by each individual physician, the results are compared and discussions are held in an effort to standardize the criteria. The number of subjects examined during the trial period is gradually increased so that perhaps by the 3rd or 4th day all the clinicians are doing complete examinations on each of 15 or 20 individuals. The number of subjects examined should be increased so that the flow of examinees will approximate actual field operations.

In numerous surveys it has been demonstrated that one of the most important sources of confusion and differences in physical findings between groups is the individual examiner's idiosyncrasies of definition and recognition of clinical signs. Accordingly, it is essential to eliminate or at least to minimize these variations. Hence, the necessity for clear definition and standardization of observations cannot be overemphasized. The definition of clinical signs to be recorded is a matter of joint decision by the nutritional examiners who should, however, adequately describe and record their criteria. Once this decision is made it must be adhered to by all groups throughout the period of study. After the initial standardization training period, it has been desirable to include in the survey itinerary 1- to 2-day examinations of the same individuals, if possible, at the beginning, middle, and end of the survey.

In recording the physical examination and biochemical data, it is most useful to utilize a punch card system. This requires preparation of a code so that the

data can be summarized from the cards or transferred to an IBM card. Methods of recording and coding the data have been described in detail in (6).

Often special physical examinations will be made in the field. These data and the biochemistry on these subjects should be differentiated from the random sample by the use of different numbered or colored cards.

During the advance preparation the nutritionist responsible for conducting the dietary survey should also plan a test or training operation for those individuals who have had less experience in the specific techniques to be utilized. A questionnaire based on local foods, food habits, and customs should be prepared.

SAMPLING

General. The total size of the sample needed to accomplish the objectives of the survey cannot be specified in advance. The sampling plan which is appropriate for one country may not be so for another country. There must be a balancing of the facilities of the team in the problems of travel to the location against estimations needed to evaluate nutritional status for both the total population and the appropriate strata or subdivisions within the population. For example, if the finding of any condition in the population study is to be compared with a normally expected 1% rate of findings, and if the actual rate of occurrence of this condition in the population studied is 3%, a properly drawn sample of 500 individuals would be more than adequate to establish the difference.

Every effort should be made to obtain true representation of the population by random selection. In some cases it may not be possible to do individual random sampling and it may be necessary for the sampling to be placed on a unit basis so as to obtain the most information for the given amount of time of survey. Examples of selection of population samples are given in recent published surveys (2, 3, 5, 7). An example of the procedure used for sampling the civilian population in Ecuador (4) is as follows: fifteen areas were selected to provide a sampling of the varied regions of the country and to afford an access point to nonurban groups. Civilian locations were chosen to be within a 2- or 3-hour drive by motor from the major city within each area. By use of a table of random numbers, a sample of villages and towns representative of those with different total populations and within such limits of access to the centers as required was selected. Alternates were chosen for each location and insofar as possible the villages studied were limited to the samples selected. Districts in which the two larger cities are located, Quito and Guayaquil, were selected on the basis of economic level and the major occupation of the population. Health educators and other public health personnel cooperated in the establishment of contact in the villages and assisted greatly in interpreting the purpose of the survey and in obtaining the cooperation of the population at the many points of the country surveyed. Contacts were made also through influential members of the community, members of the clergy, landowners, school officials, and teachers. Random sampling is not always possible. As would be expected, in some locations samples necessarily consisted of those who volunteered for the examination. Others were accessible at a community level through the schools. Here the sample comprised largely families of children attending the school. In certain locations, special groups of examinees were selected on the basis of previous nutrition survey work carried out in that area. In addition, school children and pregnant or lactating women were often examined as a special subgroup.

It is important to remember that the initial plan for sample collection may not be consistent or feasible with the actual working plan. It is, therefore, essential that a complete detailed record be kept of the sample size and the reasons for selection so that in the final interpretation of the data these variations and samples can be properly weighed. Once the over-all sampling plan has been developed it is essential to determine various subsamples, such as the number of individuals to receive a physical examination.

Physical examinations and sample. If large numbers are included, it has been found advisable to conduct two types of physical examinations, one an abbreviated type in which the major physical indicator lesions of nutritional significance are recorded (see fig. 1) and the other a more detailed examination. With a team consisting of members as previously described, it has been found feasible to examine from 300 to 500 individuals daily with the abbreviated type of examination, with every 4th or 5th individual who receives the abbreviated examination also being given a detailed examination. This procedure in itself furnishes excellent data for comparing consistency or variation between clinicians on a fairly large number of individuals examined at least twice. (Charts describing the flow of subjects through the examining line are shown in figs. 2 and 3.) The survey team in Ecuador operating in the field approximately 65 days examined a total of 2,279 members of the Armed Forces and a total of 4,876 civilians in 15 areas.

Biochemical samples. Biochemical facilities are usually not sufficiently extensive to enable the analysis of blood and urine from all individuals receiving a physical examination. In general, 1 out of 4 or 5 of those individuals receiving a detailed physical examination is selected for collection of blood and urine specimens and parasitologic studies when feasible.

Biochemical methods. The biochemical methods selected should be those that have been adequately tested for reliability and ease of operation. It is not always possible to use the most detailed, precise method. However, those methods used in past surveys have been selected for their reliability and adaptability for field studies. The collection of samples in the field requires considerable advance planning. In examination of civilian populations it is usually only possible to obtain a random blood and

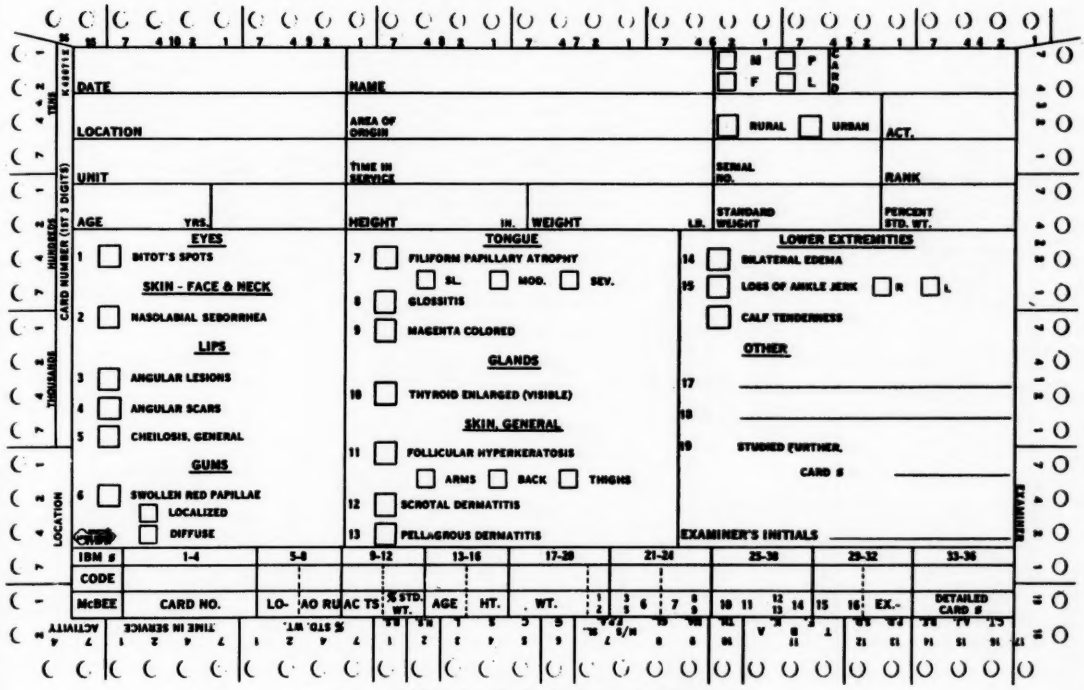

FIG. 1. Abbreviated physical examination card.

urine sample. Blood samples are most conveniently drawn by the use of vacutainers. The usual procedure is to draw 2 samples, 1 oxalated 5 milliliter sample and another 20 milliliter sample not oxalated. There is a tremendous advantage in shipping blood and urine samples back to the base laboratory for analysis. In most areas throughout the world arrangements for air transport have been possible, enabling samples to be returned to the laboratory within 24 to 36 hours. With proper precaution in refrigerating and stabilizing special samples, this time interval does not materially influence the analytic results. Capillary blood specimens taken for microchemical determinations where indicated, such as from children, likewise can be refrigerated and returned to the laboratory.

In general, the following biochemical determinations are carried out on the blood samples: serum protein, and where indicated separate special determination of serum albumin and globulin; hematocrit; hemoglobin, serum vitamin A, carotene, and vitamin C. Urine samples are usually analyzed for thiamine, riboflavin, and N′-methylnicotinamide. If cholesterol determinations are desired, plasma dried on filter paper can be held for later examination. For parasitologic work, blood smears are made on microscope slides, dried, and shipped for staining and reading. Fecal specimens are prepared in the field by the merthiolate, iodine, formalin procedure (1). The laboratory work load should have some flexibility so that special analyses may be conducted as the problems arise.

Dietary sample and methods. The dietary food intake studies should be conducted on those subjects receiving a physical examination, and where a questionnaire approach is used, it is desirable to interview those individuals who have also been given the biochemical examination. In the Ecuador survey (2) dietary information was obtained through a standard detailed 7-page questionnaire for the purpose of recording information on family food habits, foods used during the previous 24 hours (questionnaire recall), weekly food purchases, frequency of use of different foods, special dietary practices for infants and children, and other pertinent information concerning the composition of the family.

Time and economic factors may sometimes dictate the time allotted for dietary surveys at the various survey locations. A satisfactory sample size for dietary appraisal will depend upon many factors. It would be desirable to survey samples representing not less than two tenths of the people in the country with a total population between 3 to 5 million. A smaller percentage for a larger population and a larger percentage for a lower population may prove satisfactory.

Experience in the survey of the civilian population in Ecuador in 1959 has shown that a relatively small number of personnel can obtain sufficient data by more than one method of dietary surveys to permit checking the results of one method against another. A questionnaire recall method was conducted on 341 families representing a total of 2,087 individuals. Of this number 28 families (184 individuals) were further studied by 2 other methods; namely, the recipe method in which food

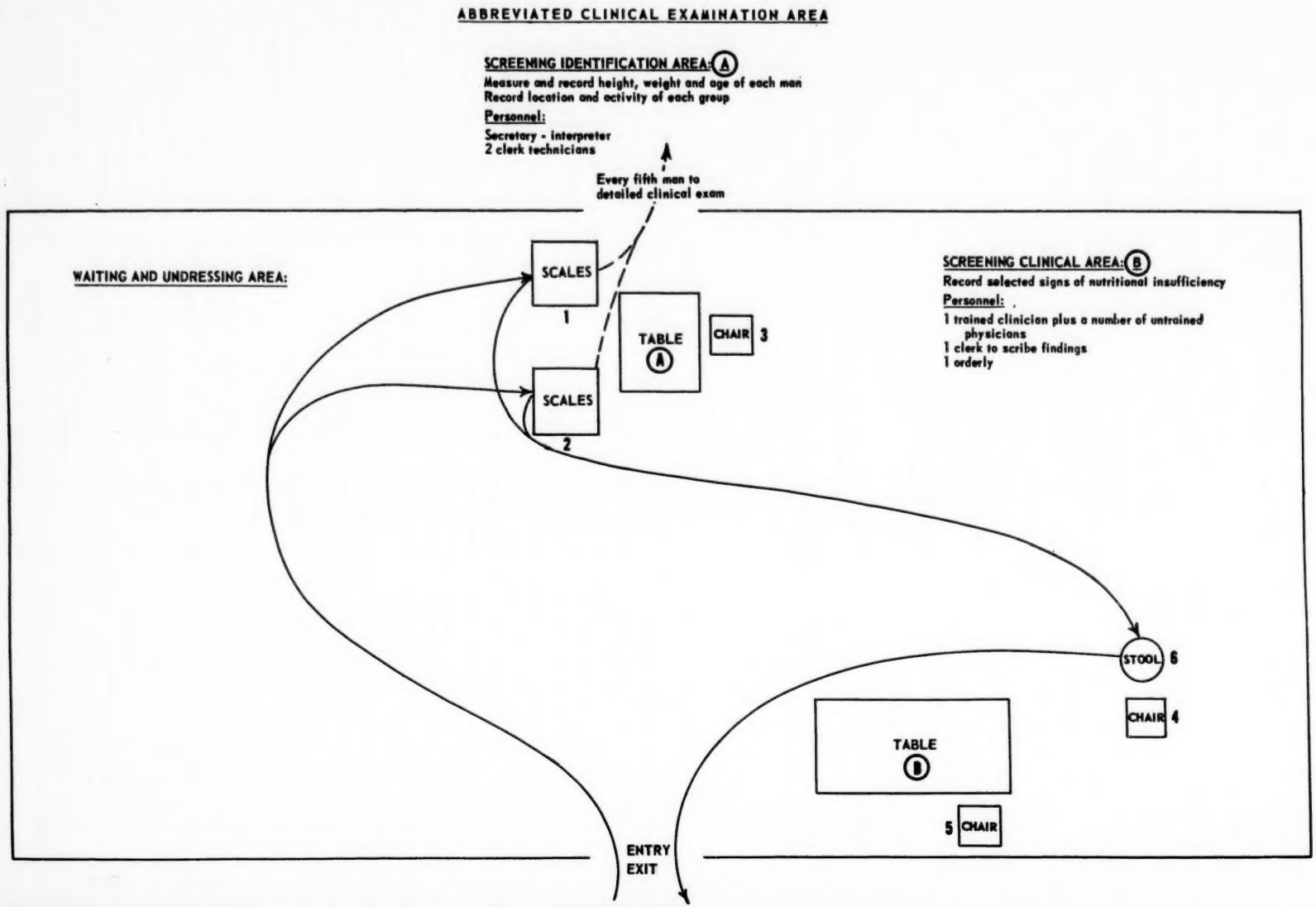

FIG. 2. Abbreviated clinical examination area.

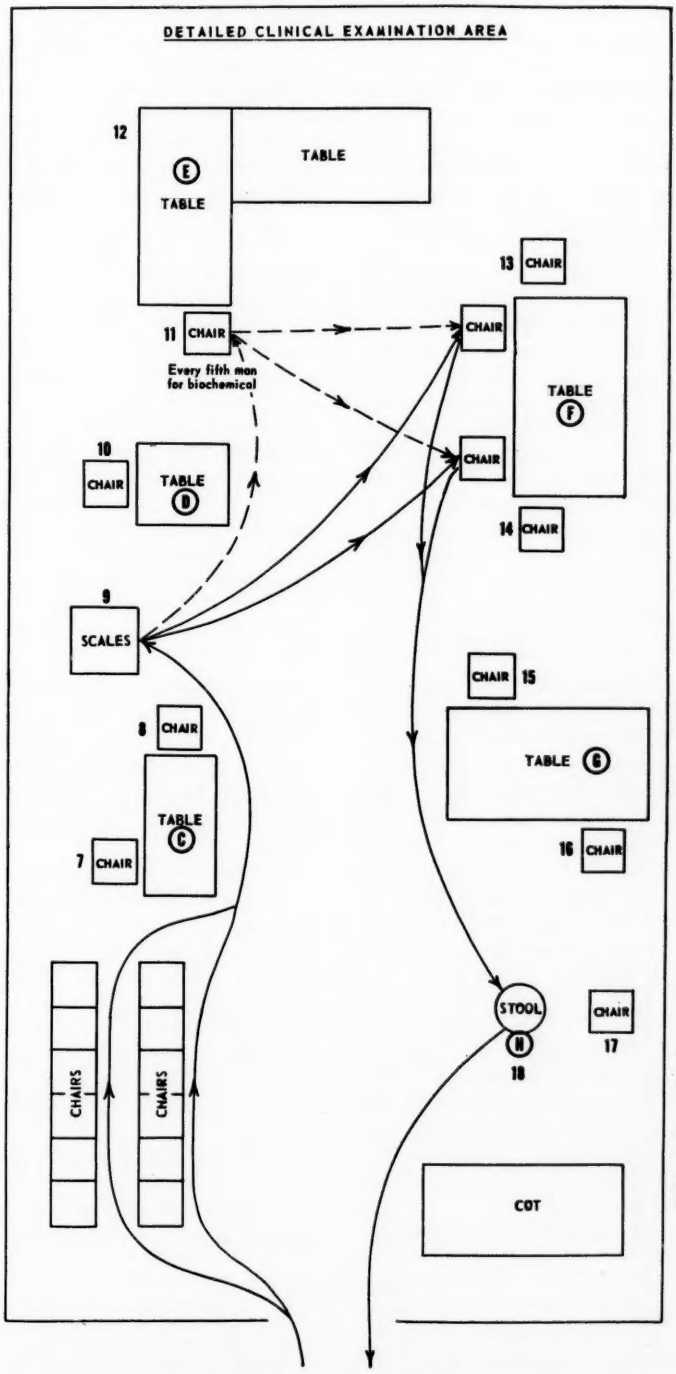

FIG. 3. Detailed clinical examination area.

FIG. 2. Abbreviated clinical examination area.

consumed by the family was systematically weighed and recorded and the food composite analysis method in which a representative sample of the average daily food consumed is obtained and prepared for direct chemical analysis. These civilian data were obtained concurrently with surveys in 9 military installations of the Armed Forces during the 2-month period of the survey. The results of the 3 methods, questionnaire, recipe, and food composite analysis, were in sufficient agreement to establish confidence in the procedures.

Dietary surveys in military installations or institutions are relatively simple compared to home family studies, for in the former instance one is able to conduct the study for longer duration, weighing and recording accurate weights of the various foods that are consumed. At the time of the survey it is also important to obtain information regarding food habits, customs, seasonal variations, etc. In many of the countries where there is little variation between meals from 1 day to the next and even between 1 meal and another, the time interval necessary for dietary appraisal is greatly reduced. In some instances 1-day surveys will adequately describe the average usual nutrient intake. It must be kept in mind that for a better appraisal of the dietary intake, it is extremely desirable to have repeated surveys at different periods of the year based on seasonal availability of foods. Analysis of food composites has numerous advantages. One is that it enables fairly precise estimations of the nutrients actually consumed, in which cooking losses have already been accounted for. It also takes into account variations of nutrient content of the various foods, and provides means of estimating other nutrients that one cannot calculate from the usual food composition tables now employed, such as pantothenic acid, vitamin B_{12}, pyridoxine, fluorine, iodine, total sodium chloride, etc.

Agriculture and food technology study. The agriculture and food technology studies are an important part of the survey. In many instances this phase of the survey may be conducted separately from the work of the field team. However, whenever possible, the food technologist performs many duties in the field which are of great assistance and value to the dietary group, such as identifying special or unusual foods, determining types of home-processing and surveying the potentiality of further development of food technology within the area. These points are of vital importance in formulating practical recommendations following the survey.

Ancillary data. The field team has the added responsibility of collecting supplemental data regarding health statistics, agriculture, etc. This is usually accomplished by visiting the various hospitals and institutions within the area. It is especially important to realize that the severely malnourished individuals may often be hospitalized. Thus, the field plans should include time to enable proper consultation of the clinical group with the local hospital and medical profession.

Preparation of the report. Plans for tabulation of the data should follow the procedure as specifically stated in the initial plan. The most difficult phase of a survey is the interpretation of the findings in which the 3 methods of assessment, physical, biochemical, and dietary, must be integrated. Guidelines for interpreting the biochemical, dietary, and physical findings are given in reference (6). After the team has prepared a preliminary report, the findings should then be discussed with the group that assisted in the initial planning of the survey. Recommendations should be adequately discussed and their implications outlined with the various governmental agencies that may be affected. This may include changes in agricultural programs and may involve an education program for specific segments of the population. It may include long-range programs for the agencies involved in economic development. In most cases it will involve continued follow-up work. The initial survey is only the beginning. If a major nutrition problem is indicated this certainly should be further studied by additional techniques, such as therapeutic and preventive test trials, so as to insure the specificity of the conclusion and indicate the practicability of preventing the disease.

SUMMARY

One can only justify the reason and need for conducting a nutrition survey on the basis that it will eventually assist in improving the nutritional health of the population concerned, and that due to the inherent techniques employed, the findings contribute toward better knowledge and understanding of human nutrition.

In conclusion, I wish to encourage the following:

1) That in nutritional assessment interpretation of the findings be based upon the 3 methods of appraisal, physical, biochemical, and dietary, until such time that we have sufficient evidence to indicate that one of the procedures is specific enough to clearly identify the major problems.

2) That all proposals for nutritional improvement be chosen carefully for adaptation to the geographic, economic, and cultural pattern of the population concerned.

3) That initial immediate programs for improvement be based on utilization of local resources.

4) And finally that we still have much research to do and much to learn as regards optimum nutrition.

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Public health indices of the nutritional status of man

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PUBLIC HEALTH is a comprehensive conception. Winslow defines it as follows in the book of Simmonds and Kinsey: *Public Health in the World of Today:*

"Public health is the science and art of preventing disease, prolonging life, and promoting physical and mental health and efficiency through organized community efforts for the sanitation of the environment, the control of community infections, the education of the individual in principles of personal hygiene, the organization of medical and nursing service for the early diagnosis and treatment of disease and the development of the social machinery which will ensure to every individual in the community a standard of living adequate for the maintenance of health."

This definition presents a further elaboration of the verdict of the World Health Organization: "the attainment by all peoples of the highest possible level of health." It defines health as a state of complete physical, mental and social well-being, and not merely the absence of disease or infirmity.

If one accepts these two definitions, it is obvious that the objectives of public health are broad and that the attainment of them is a matter of primary importance to the entire human family. It can be established without any trouble that nutrition has to perform an important part within the framework of this description.

The attainment and maintenance of a proper condition of health for a human being living in a community comes entirely within the sphere of public health. To illustrate this, it will suffice to state that it is not imaginable to maintain a proper status of health, to prevent disease and to prolong life without proper nutriment.

It might have been better if in the definition of Public Health the words "promotion and maintenance of an optimal status of health" had been substituted for "the maintenance of a proper status of health."

From the definition it is also clear that public health, as far as nutrition is concerned, does not in the first place take care of the individual but rather of the status of health of the community. Public health indices will have to give an idea of the nutritional and health condition of groups of the population, eventually of the population as a whole, at a fixed moment or in the course of a period. Data collected only once for this purpose will rarely enable us to give a correct interpretation of the actual conditions prevailing. The status of health of a population is not stable but always fluctuating. In addition most criteria render by themselves information too incomplete for an unequivocal explanation. Comparison with the data recorded in previous and subsequent years will always be necessary. Over and over again we will be confronted with the situation of the past and with what is expected. Comparison of results relating to different social groups will often be necessary, and the same applies to what is to be found abroad, in order to assess the criterion concerned at its true value.

Some statistical data on health, infirmity, or mortality, to be discussed later, may in this sense render an indication with regard to the nutritional status of the population. Data on health only, however, have a limited significance as nutritional indices to public health. Besides data on consumption will have to render supplementary information.

An anticipation of the nutritional status of a people or of a group of people can be fairly derived from nutritional data relating to the people or group concerned, if its composition according to age classification and occupation is known. For a number of countries it is possible to calculate a rather correct consumption average from data on production of articles of food and on imports and exports after corrections for the quantities not intended for human consumption. If judged according to the probable need of the population of calories and nutrients, a certain assumption in respect of the state of nutrition of the population can be arrived at.

To complete these data and to get a better interpretation, budget investigations within groups of the population are of some importance. As a matter of fact it is quite imaginable that the average consumption is at a reasonable level in a country, though it leaves very much to be desired within some groups of the population.

The nutritionist occupying himself with matters of public health will not be satisfied with data on consumption only. Apart from nutritional data he wants data on

the conditions of health, which also demonstrate the most direct connection with nutrition. Such data are difficult to come by and therefore he will have to be content with data on health, infirmity or mortality, of which a relation to the nutritional condition can be assumed.

General death-rate data will give an idea of the general status of health of a people, though that status may already have altered at the time the data become known. In addition, these data have the drawback that they apply to final conditions and are always issued too late. They never are a timely signal that a threatening decline of the status of health is developing. Moreover, indices of mortality rarely supply direct information on the nutritional status.

More suitable for the purpose are figures on diseases and mortality caused by tuberculosis. A connection between public nutrition and the frequency of cases of tuberculosis among the population is now generally accepted. Results of various investigations, in particular among young people, show that the susceptibility to tuberculosis increases if nourishment is inadequate. Which nutritional factor or factors are playing a part here, either directly or indirectly, is not known with certainty. Though 'poor feeding' caused the susceptibility to tuberculosis to increase, it stands to reason that other factors, such as the risk of contamination, are of much importance. It is a fact that the number of cases of tuberculosis is highest where the nutritional conditions are poorest. War conditions in Europe have revealed that the number of cases of tuberculosis and subsequent mortality rose substantially when the general conditions became worse. That the higher risk of contamination is not alone responsible for the increase of tuberculosis became plausible by the events in England. In that country mortality due to tuberculosis decreased with the improvement of the nutritional conditions, in spite of the worsening of hygienic conditions and the increased strain.

The following graph taken from *Child Mortality in the Netherlands* by de Haas (2, 3), shows that, e.g., in the Netherlands the general picture of causes of death has very much changed during the last 30 years (fig. 1).

Infections of the bronchial tubes and tuberculosis are now causes of death of secondary importance and are no longer the main causes of mortality. The pattern of causes of death has entirely changed.

In the framework of this paper it is of importance to state that mortality due to tuberculosis within the group of adolescents to whom the graph relates has considerably decreased. During World War I (1914–1918) as well as during World War II, the mortality due to tuberculosis rose rapidly. During the First World War the rate of increase was 60% and during the Second World War 90%, though in the latter period when extremely poor conditions prevailed the total death rate was considerably lower than during the first period. After the

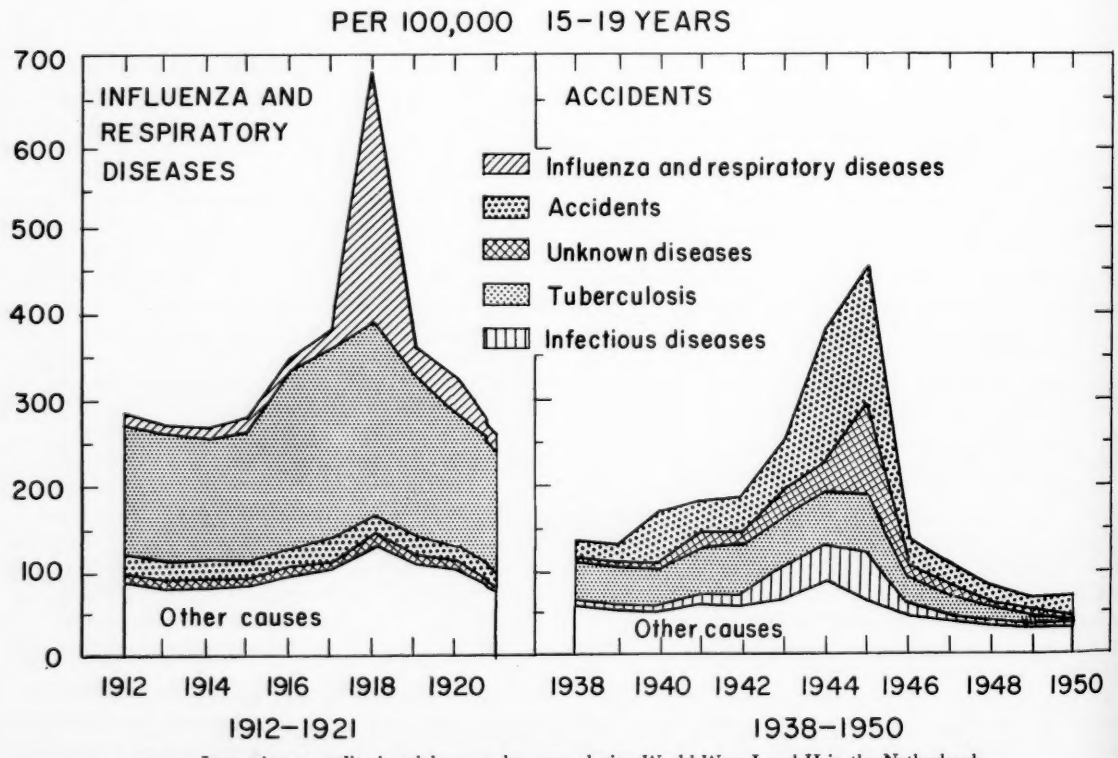

FIG. 1. Increasing mortality in adolescence by cause during World Wars I and II in the Netherlands.

Second World War a rapid decrease in mortality due to tuberculosis below the pre-war level was noticeable, a fact much less pronounced after the First World War. This difference in behaviour of tuberculosis after both wars can, in my opinion, be explained by the higher resistance the population had acquired by improved commonly adopted nutritional habits during the period just before the Second World War broke out. This will most probably also explain the big difference in mortality on account of infectious diseases during the first and the second world wars. A graph compiled in the same way for infants produces a similar picture as the graphs drawn up for adolescents.

An increase in the number of cases of tuberculosis within the groups susceptible to the disease may also be an indication of a worsening of the nutritional conditions. Several arguments can be put forward to explain the fact that tuberculosis is a more frequent complaint among adolescents and young adults than among other age groups. A quick rate of growth of the adolescent, linked up with great activity, demands high standards of nourishment. Due to this and also on account of the developing feeling of independence with the consequences involved in respect to nutritional habits, the risk of inadequate nourishment in this group is much bigger and predestines them to an inadequate resistance to contamination.

Some Dutch data are set out in the following table:

New Cases of Tuberculosis per 100,000 Inhabitants, by Age

	0–1 yr.	1–4 yr.	5–14 yr.	15–19 yr.	20–29 yr.	30–39 yr.	40–49 yr.	50–59 yr.	60 yr.
1949	58	115	159	262	*314*	183	138	127	94
1953	7	55	175	164	*205*	162	115	100	90
1958	8	30	75	76	175	94	72	66	66

Figure 2 also clearly portrays this feature. In the mortality statistics (fig. 3) this increase among the adolescents is not noticeable. This is most probably due to the increasing general resistance within this group.

The resistance to injurious external conditions is smallest with babies. Improper nourishment will have the worst effects within this group. In some countries, the influence exerted by wrong feeding on mortality of babies now almost belongs to the past. It was in these countries that diarrhoeas especially in summer due to wrong nourishment caused the high peaks in the mortality of babies. Figure 4 clearly reflects this statement. The fall in mortality during the summer months experienced today has been attained by a proper enlightenment on nourishment and general hygiene during the baby stage.

In 1952 Gorter wrote: In the Netherlands the mortality among babies is no longer the most important indication of their health but rather of the nature of the causes of the mortality among children. The official

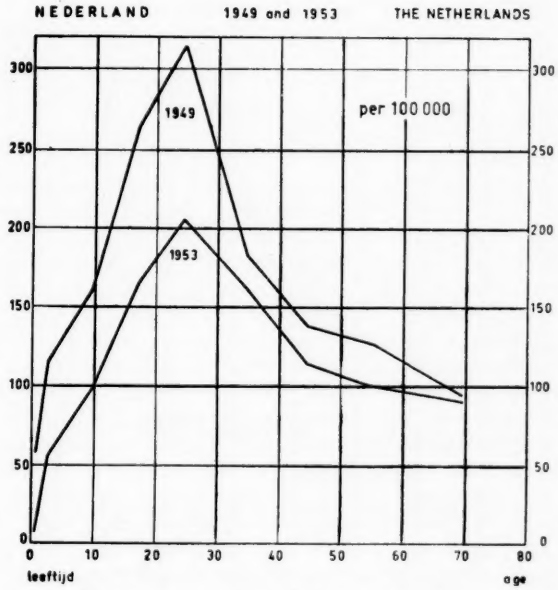

FIG. 2. New cases of tuberculosis by age groups (De Haas).

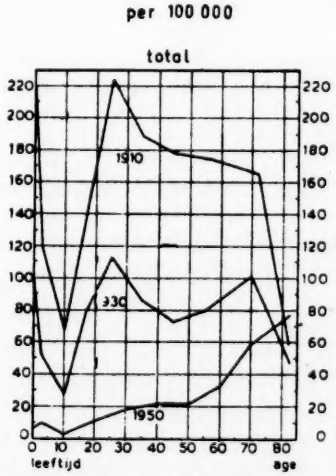

FIG. 3. Death rate from tuberculosis by age in the Netherlands (De Haas).

statistical data on this subject are extremely unreliable (according to Debré less than 40% reliability). In 1927 the official Dutch data were verified. In those areas, where mortality among babies was highest, the official data were most incorrect.

As soon as the decrease in infant mortality has attained the level of 3.6–3.8%, the ratios of causes of death show that the termination of nutritional disorders has been the first to contribute to this decline. The same applies in a lesser extent to infectious diseases. In a period of high mortality the number of nutritional disorders is also higher again, such as with cases of diarrhoea, cholera infantum and dystrophy. Infant mortality shows regionally

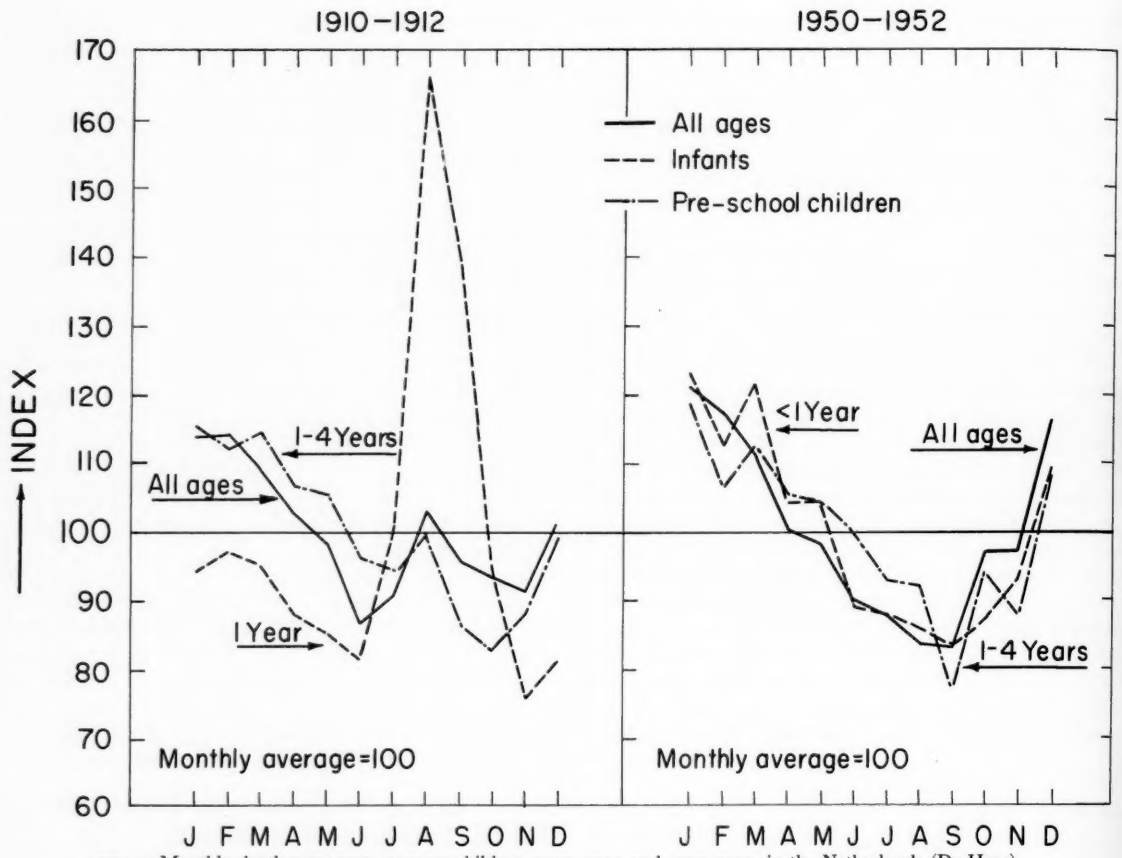

FIG. 4. Monthly death rates among young children, 1910–1912 and 1950–1952, in the Netherlands (De Haas).

big differences as is revealed by the map in figure 5 of the Benelux countries and Western Germany.

High infant mortality implies in general wrong infant nourishment just as anaemia in babies points to the use of an incorrectly composed diet. Often the diet of prospective mothers during pregnancy is already inadequate. Only in exceptional cases will the hemoglobin content be determined in babies. Unless an investigation on this phenomenon is intentionally carried out, it will be difficult to use this datum as an index of the nutritional status. In countries where confinements take place in hospitals it is in better use. This also applies to the frequent prevalence of premature births.

Baird in England showed that premature births, the number of stillbirths and mortality during the first 2 weeks of life were 2, 3 and 5 times as high among primiparae who as girls had grown up in less prosperous environments than those girls who were from more prosperous classes. Also data on premature births, as a criterion of the nourishment of pregnant women, are not easy to come by.

In several countries, however, an analysis of perinatal and infant mortality will be possible. An analysis of Dutch data shows that since 1949 the number of stillborn babies, deaths during the 1st week after birth and mortality among babies after the 1st week has steadily decreased. It also appears that mortality is not the same in all provinces.

In the provinces where mortality is higher, mother and child are not so well looked after but the obstetrical aid in the various provinces shows little or no difference. The nutritional conditions are undoubtedly not equal in all provinces. A high percentage of premature births is responsible for the high rate of death during the 1st week after birth. In 1955 it was still 20% of the total infant mortality.

As regards the stillborn babies, the cause in not less than about 16% of the cases is toxemia during pregnancy. Undoubtedly there is a close relation between nourishment and toxemia, although the exact connection is still insufficiently known. In the Netherlands 1 woman of 3 shows slight symptoms of toxemia. Thanks to quick action these symptoms are seldom allowed to develop into a serious form of toxemia. One may readily assume that there is something wrong with the nutrition of pregnant women and that an inadequate diet is also responsible for this symptom.

Also infants are susceptible to wrong and inadequate feeding. Bongoa is of the opinion that the ratio of mor-

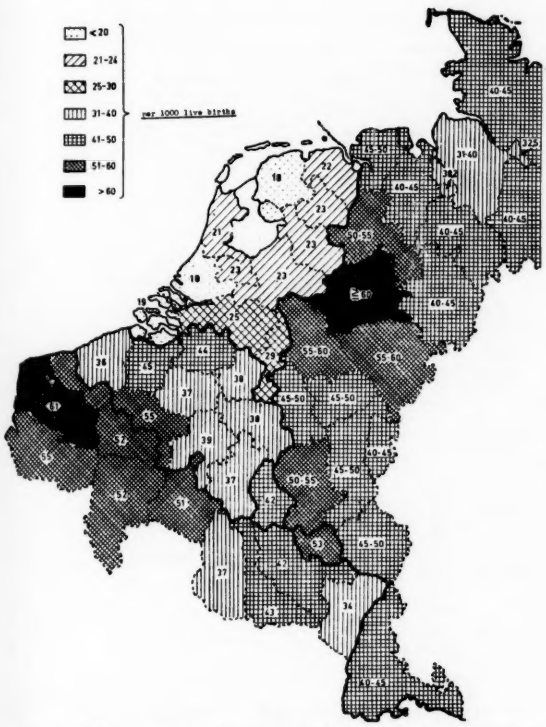

FIG. 5. Infant mortality in the Netherlands, Belgium, Northern France and Western Germany by province or department.

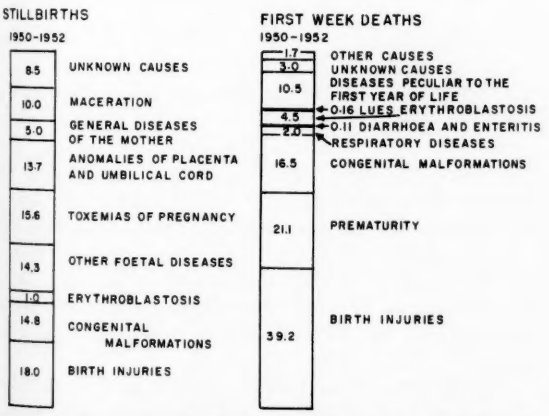

FIG. 6. Percentage distribution of perinatal causes of death in the Netherlands (De Haas).

tality among children of less than 5 years of age and the total mortality is a useful index of the nutritional status of a population (4). These data are comparatively easy to collect, as no complete statistics of mortality are required for the purpose. It will suffice to make random tests in a given area.

In Western countries and the United States of America inadequate intake of calories can lead to a poor nutritional status within somewhat larger groups. Undernourishment will remain restricted to incidental cases.

The cause of the effect of nourishment on the development of the deviations of health referred to above or on the shortening of life must be attributed to a wrong qualitative composition. Also caloric over-nourishment may lead to frequently occurring disturbances.

Genuine deficiency diseases rarely occur in countries with a high standard of living. Usually goitre and rachitis are most frequent. Due to the many prophylactic measures serious forms of goitre seldom occur anymore and it is often the areas where slightly enlarged thyroid glands are common that are overlooked now and then. A systematic investigation can only reveal whether an iodine prophylaxis will be sufficient. An investigation on the size of the thyroid glands particularly in children is of value only if the examination is performed by one and the same person according to a standardized method. It may be that the examination can be properly performed by several physicians, provided that they are well instructed and that the method as applied by them is carefully kept under control by mutual comparisons.

Very likely the goitre-frequency examination as an index of iodine efficiency can be entirely omitted, if the drinking water is systematically tested for its iodine content. If the iodine content of the drinking water is less than 40 μg per litre, an extra supply of iodine for the area concerned is necessary. It can be assumed in that case that the supply of iodine to the population is deficient.

Rachitis is becoming a disease less frequently met. Continuous random tests on the frequency of this complaint, however, must be made in order to make sure that an often suboptimal supply of vitamin D is not turning into an actual deficiency.

In countries with a high standard of living the suboptimal conditions of nourishment, as the cause that life does not fully develop itself, are usually difficult to identify. This will often result, in children, in an unsatisfactory development, both mental and physical, increased absenteeism from school due to illness, and more applications for holiday camps, etc. Adults often lack sufficient love of work and their achievements remain below those expected from them. The diagnosis of a suboptimal status of nourishment is extremely difficult and cannot be made prior to an extensive examination, clinical as well as biochemical. Such a comprehensive and expensive examination is only justified if there are sufficient indications that it is absolutely necessary. These indications must be regularly looked for.

The general opinion in the Netherlands is that the following system should be applied. With the cooperation of school physicians a census is taken every year of the food consumption by school children and of their growth and development.

In consultation with the statistician it is determined which children will be included in the investigation, well dispersed over the country according to town, village and rural district. It has been decided to choose children 8 years of age. One or more centrally appointed dietitians record a simple but exact nutritional anamnesis of

the children. The nutritional anamnesis is simple in this respect that mainly inquiries are made with regard to the articles of food supplying animal protein and contributing substantially towards the supply of minerals and vitamins to the child. As a rule these are also the most expensive nutrients in the packet of articles of food. Furthermore weight, height and sitting-height and also the thickness of the subcutaneous fat are determined.

The results are given as frequency divisions per quantity group for milk, arranged according to a given system (e.g. consumption of milk in quantities per day: $<\frac{1}{4}$ litre per day; $\frac{1}{4}$–$\frac{1}{2}$ litre per day; $\frac{1}{2}$–$\frac{3}{4}$ litre per day; $>\frac{3}{4}$ litre per day). A division is also made for cheese, meat, vegetables, fruit and eggs. Height and weight, sitting-height, etc. are recorded per age class and also expressed in percentages. The data are compared from year to year. The assumption is that if the economic conditions get poorer, the decline will immediately be reflected by an unfavourable effect on consumption particularly of the more expensive articles of food and, at the same time, that the children will react to this situation by a decrease in weight and in the long run by a slower growth. If the tendency described here is evident, the time has arrived for an extensive nutritional investigation.

The data on nourishment recorded by the centres for prenatal care are assembled centrally, the same train of thought being pursued here. An economic decline will be reflected in a change in consumption, particularly of the dearer commodities. The data on nourishment from the centres for prenatal care are collected and worked up at all the centres in the same way. Also here the nutritional data can be followed from year to year.

It is clear that this repeated transversal investigation, especially if the group to be examined is a diversified one (infants, school children, pregnant women) may render a proper idea of the nutritional status of the population.

With regard to the development of children, also a proper picture of the secular shifts will be obtained. Determination of the annual rate of growth of children is very likely of much value. A decrease in the annual rate of growth must be considered as a danger signal. School physicians are often able to supply these data. To get a proper idea of the nutritional status of a group one single fact does not usually suffice. On the other hand, however, simple data may render valuable information. Low values of vitamin C in the blood for instance may point to the fact that the consumption of vegetables has been low in the past.

In investigations on nutrition and conditions of health it would be desirable to apply methods rendering results which are satisfactorily correlative. In many cases the data recorded by anthropometric, biochemical or clinical methods of investigation are in conflict (5).

Indices on the nutritional status of adults are difficult to obtain. In the western states of Europe and in the USA real deficiencies are rarely encountered. Subclinical deficiencies are more difficult to identify in adults than in children. As a rule the adult in these

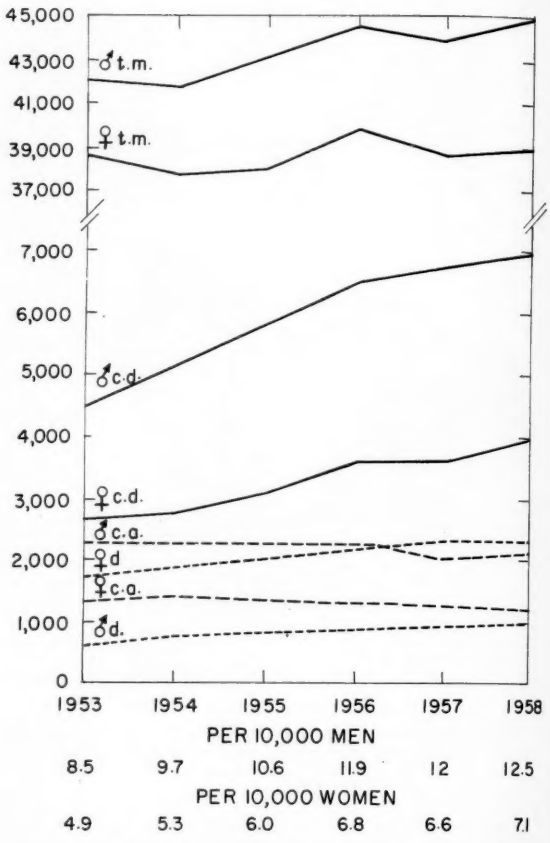

FIG. 7. Mortality from heart disease (c.d.), stomach cancer (c.a.), diabetes (d), and total mortality in the Netherlands, 1953–1958 (Van Buchem).

countries is suffering from overfeeding, resulting in an adverse effect on his health. To determine slight overfeeding is as difficult as to prove a subclinical deficiency.

Unmistakably associated with nutrition are atherosclerotic changes in the blood vessels which may be a secondary cause of death. The real cause is undoubtedly a complexity of factors, among which nutrition is probably a decisive one.

Considering the subject of this paper it is not opportune to start a discussion on the question of which nutritional factor or factors should be held responsible for atherosclerotic lesions. In most countries with a high standard of living, morbidity due to atherosclerosis increases. This should be a more sensitive criterion as a public health index in connection with nutrition than mortality due to atherosclerosis, but it is difficult to get an exact record of it.

Figure 7 shows the change in mortality rates for these causes of death (1). From 1953 to 1958 the number of deaths caused by atherosclerosis among males rose from 8.5 to 12.5, per 10,000 men, and for women from 4.9 to 7.1 per 10,000. It may be that increased mortality is

not as high as it seems. Statisticians believe that the increase is 20% instead of 50%. While from a number of cases of heart diseases it is not certain that these are of atherosclerotic origin. It may be that the change in these figures is a significant indication of the prevalence of certain diseases among the population due to overfeeding.

In general it can be stated that the public health indices intended to show the nutritional status of a population are only seldom so simple, that the part played by nutrition is abundantly clear. This applies in particular to countries with a high standard of living. Less difficult is the situation in those countries where actual deficiencies occur.

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Clinical evaluation of calorie and protein status

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MANY ILLNESSES, above all chronic diseases, go hand in hand with a reduction of the general nutritional status. A lot of them show in their still undiagnosed initial phase a loss of weight, so it is natural for the clinician to be asked how to make a general objective appreciation of the nutritional status of his patients. When, however, one wishes to satisfy oneself beyond the purely visual impression, for instance, by determining body weight, difficulties are already met with.

The ordinary weight tables of the life insurance companies or official health organizations naturally take hardly any notice of anthropometric measurements and represent average figures which do have a certain range and are valuable as a primary orientation. The nutritional status as such, however, is only dealt with in rough outlines, without defining in a really exact scientific manner the constitution of the organism concerned. Many factors influence in opposing directions the primitive figures of the body weight—I only need to mention a virtual depletion of cell mass in consequence of too highly developed fat or the appearance of water retention in cases of protein deficiency, and so on. This easily happens for instance in both examination of a single case and in large-scale communal surveys, in which connection I should like to mention a small point of personal experience. During 1931, at the time of the deepest economic depression in Europe, the League of Nations endeavoured to form a picture of the effects of unemployment upon those sections of the population which were concerned. A commission consisting of hygienists, social welfare physicians, and clinicians met in Berlin in an endeavour to start a common movement in this direction in the various countries. The most obvious method, a simple check on weights of a group of the population over a certain period, taking into consideration the nutritional conditions pertaining to the income of the unemployed and their families, broke down the very serious doubts expressed by the hygienists who thought that such a procedure was burdened with too many sources of error. And we parted in a state of dissatisfaction, with the Berlin clinician, von Bergmann, remarking resignedly that only on the dissecting table can undernourishment be proved.

In our attempts to find the methodical basis for determining a nutritional status, we can certainly at the present time turn to a large number of methods of appraising this in many ways and thus objectively understanding it. If, in my lecture, I often refer to the difficulties of the clinician, I must ask you to forgive me; for sick people, in particular, cannot be subjected to every method of examination which perhaps could be applied, without any trouble, to healthy persons or those suffering only from malnutrition.

It also would not be necessary to make all these diagnoses in a clinic in every case of sickness because, after all, all or most illnesses are accompanied by interrupted intake of nourishment, as a result of lack of appetite or by reduction of cell mass as a result of catabolism, and only certain metabolic diseases require careful control of the nutritional status in addition to their contingent specific therapy. As Berryman very rightly stresses in his excellent survey of "Nutrition—and Nutritional Diseases" in the *Annual Review of Medicine* (9), such a survey can only be an outline and will consist principally of omissions.

The general diagnosis of nutritional status to a great extent corresponds to the general calorific status and after the weight has been reviewed in relation to certain physical constants, such as height and perhaps general physical build—athletic and muscular, asthenic, pyknic, etc.—a closer description is to be recommended by measuring some skin folds with reference to the thickness of the subcutaneous fatty tissue. According to a "Manual for Nutrition Surveys," measurement of the thickness of the skin is advisable, a process which has been used by Keys (21) in the U.S.A., in Germany by Lauter (24), and especially by Edwards (15) in England, in an extended form with numerous fixed points of measurement. The layers of subcutaneous fat, which can be in part photographed by tangential x-ray of the predilect parts of the obese, may be the nates, the flanks, the abdominal wall and the upper thighs, while Edwards (15) in 1951 even made out a catalogue of 53 parts of the body. In contrast, according to the details in the "Manual", in practice the measurements are restricted to the scapular region and the lower axillas. In a high degree of malnutrition, in my opinion, the nates are a very good guide, in that the atrophy of the buttock muscles represents an index for widespread muscular destruction, which was used by Russian army doctors in particular to determine the degree of dystrophy.

The special composition of the human organism has only recently become the subject of detailed examination. While in the clinic, obesity is of especial interest, particularly the estimation of the fatty tissue to which we shall return later, in physiology, the determination of the whole with the skeleton (about 4–6 % of the total weight), the cell mass, the extracellular fluid and the fat mass has nowadays become a rather general method. As there are many variations of this method, we can only discuss its basic principles. It would naturally be an admirable aid for estimating nutritional status, similar to haemoglobin content, basal metabolism, etc., for providing exact figures of the actual composition of the body.

Behnke, Feen and Welham (8) developed such a method of measurement in 1942, in which they placed their men under water and determined the volume of the displaced water. A certain difficulty is presented by the various gas content of the body, which is to a great extent excluded by the determination of the air collected in the lungs with the help of the dilution of the nitrogen method in accordance with Christie (13). Keys and Brožek (22) used these tests on a large scale during their well-known malnutrition research.

Remarkably enough, such measurements, of a large group of persons, are available from the Far East and were published by Allen (3) when, together with Consolazio, Jolliffe and Pollack (14, 20, 31), he made a series of examinations in Formosa. This author, determining the specific body weight of several hundred Chinese, found a constant relation between specific weight and blood volume, whereby there was a considerable difference between men and grown women.

Some doubts must be expressed about the clinical use of the Archimedean principle of measuring specific weight by immersing the patient in a bath.

There should be a discussion in our circle as to what extent and for what patients or group of persons such measurement methods could be of importance for a clinic. Other methods of measurement use factors which are rather to be determined from sick people, for instance the extracellular fluid, total water content of the body- and blood-volume, from which, with the help of numerous formulas, computations of the cell mass, amount of fat, etc. can be obtained.

Moore and co-workers (29) in 1956 combined methods, mostly based on the dilution principle and the distribution of test substances in certain spaces, and spoke of a 'multiple simultaneous method.'

In view of the enormously different behaviour of the various components of the body towards each other, it would be necessary to determine these relations first in a large group of people. For instance, the young athlete has very little fat (10%), but even a 25-year-old man of sedentary habits shows an increase in fat to 25%, as research by Ryan and co-workers (32) showed.

The amount of extracellular fluid which is also easily determined in the clinic, if for instance the thiocyanate method is used, is fairly in accordance with an average nutritional status at 17.6% ± 2.2 of body weight. It reduces with increasing excess weight because pure fat tissue contains little water, as is well known, in spite of its considerable variability in hydration. If the patient is first of all dehydrated—which is easy to achieve with any obese patient by 3 days in bed on a diet low in salt—then with increasing excess weight the extracellular fluid is comparatively lower than normal. From what has already been said it can be seen that it is not too difficult to draw up a balance for the body, as was done by McCance and Widdowson (28) amongst others.

A very good check on the nutritional status, the general condition of a patient, also seems to me to be the total blood volume. The total blood volume also varies inversely with the specific weight of the body, which becomes less with an increasing proportion of fat. Fat tissue has less blood circulation than other organs. The formulae given by Allen (3) for men and women make it possible to compute the blood volume with considerable accuracy from body weight and the third power of height. It must be asked, of course, how far results gained from persons who are not ill can be applied to the clinic. In my opinion, here already should be a basis for the application of such formulae—at least until more experience is gathered—through the direct measurement of the extracellular space mentioned, perhaps the blood volume which is very simple to establish by means of the well-tried Evan's blue method and/or the total water by means of the antipyrine method.

If we thus have an insight into the composition of the body, then we have further methods at our disposal to achieve a general recognition of the protein status of the patient.

As even slight disturbances tend to upset the otherwise tough condition of the protein balance of the adult, a difference must be made in the determination of the actual 'protein status' (the composition of the body) that is the amount of protein, and its balance. Both should be dealt with separately.

For the evaluation of the protein status the following values in the blood are determined as minimum: *1*) the haemoglobin content, perhaps also the serum iron as reserve building material for the haemoglobin; *2*) the total protein content and the serum-protein spectrum; and *3*) the haematocrit.

HAEMOGLOBIN

The amount of the haemoglobin is a useful index for establishing malnutrition in the widest sense, even if anaemia is unspecific. In 1948 (6), in a group of 261 patients with malnutrition edema, we found the following distribution of haemoglobin values (100% Hb = 16.0 g Hb in 100 ml) (see fig. 1). In 154 patients, the distribution of the colour index was as seen in figure 2. As Goldsmith (19) says, in protein shortage there is often macrocytic anaemia—no doubt because of B_{12} shortage. The determination of the serum iron supplements the determination of the Hb in a valuable manner. The

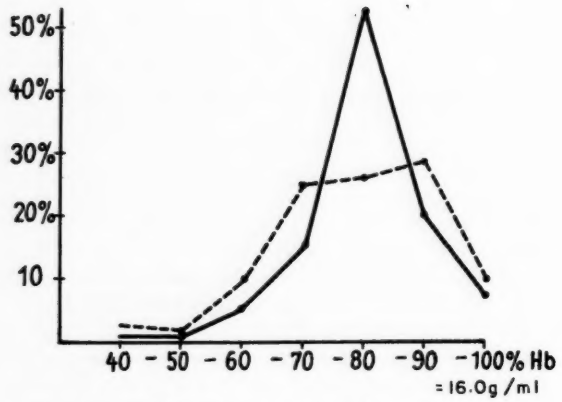

FIG. 1. Haemoglobin values: 227 men and 40 women.

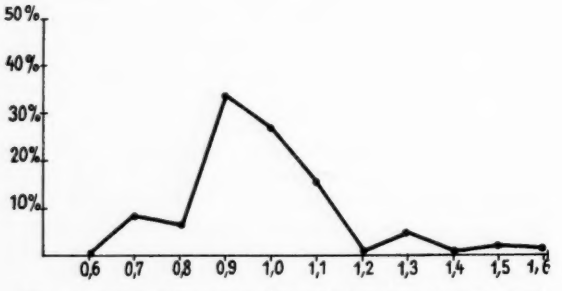

FIG. 2. Colour indices in malnourished persons (144 patients).

presence of a comparative lymphocytosis also occurs often in an insufficient protein status. The condition of the tongue and mucosa (angular stomatitis) of the mouth should also always receive attention. A glossitis and cheilosis supplement the diagnosis of anaemia in the direction of a specific shortage of vitamins and iron. A dysphagia is a most important symptom.

The amount of the serum-protein level and the composition of the serum-protein corpuscles receive a great deal of attention in the clinic, the more so as reduction of the total serum protein content and relation of the various fractions allow valuable deductions to be made. The albumin content of the serum is, of course, not an absolutely certain criterion for lack of protein; because, above all, in a deficit of protein intake which begins slowly, and/or increased protein catabolism in slow starvation or the slow devastation of a disease such as a carcinoma, the serum protein can stay quite high for a long time. There appear to be considerable individual variations, as experience of hunger edema has shown. In the continual exchange between tissue and blood, like the dynamic behaviour of all metabolic processes, there is also a continuous turnover between the serum albumin and an 'albumin pool' (*Madden* and *Whipple*, 25). Sterling (36), with the help of exchangeable albumin marked with J^{131}, found the albumin pool to be about twice as large as the amount of albumin circulating in the blood. Albumin, as so often happens, reduces more than the globulin fractions, so protein shortage edema develops in many cases as the albumin level drops. Bachet, as a result of his experiences with undernourished Belgians, gives 3.5 g in 100 ml albumin as the threshold figure for the appearance of edema. Schoen (34) and we too (6, 7) did not see any fixed limits for the development of malnutrition edema. The amount of the serum protein content is therefore of very great significance for the protein status. Elman (16, 17) and his co-workers Sachar and Horwitz (33) estimate that the loss of 1 g of circulating serum protein corresponds to the loss of 30 g tissue protein. It has been our experience that the appearance of protein shortage edema, in addition to the reduction of colloid osmotic pressure as a result of the reduced albumin, depends upon the intake of water and salt which are usually quite high in the undernourished. For this reason, important clinical signs of a state of protein shortage are nycturia and polyuria if the patients are not confined to bed. The change in the relations of albumins and globulins is also a valuable indication of protein deficiency. This albumin-globulin index is normally 1.2–1.4.

Before approaching the dynamics of protein metabolism, the serum figures for the amino acids must be mentioned. Albanese found that a reduction of the amino acid level—determined with a copper method described by him and Irby (1)—is a very good criterion of the nutritional status of an adult. He refers to observations made by Van Slyke, Man, Peters and coworkers (26, 30), who also saw in a lowering of the amino acid level a sign of deterioration of the nutritional status of pre-operative patients. Charkey, Kano and Houghan (12) observed that after 48 hours fasting, the figures of leucine and valine increased (because of increased breakdown of tissue protein) while they

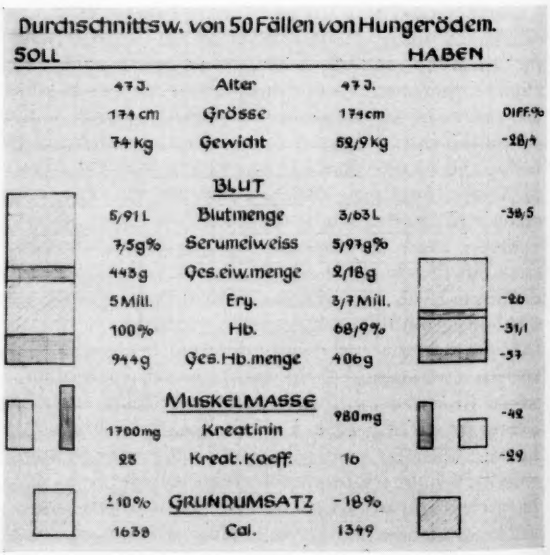

FIG. 3

dropped for threonine, lysine, methionine, arginine and tryptophane. As the fractionated determination of the amino acids has recently ceased to be so difficult, separate determination of amino acids in finding nutritional status, particularly the protein status, should become of increasing importance.

The excretion of creatinine in the urine has been of interest as long ago as Folin's discovery that the extent of the muscular metabolism is reflected in the total creatininuria. It is true that very many contradictory observations have become known but, nevertheless, the careful measurement of creatininuria has proved very useful in estimating the muscular status. It is not only used in its original sense to check the so-called creatinine coefficient (Shaffer) under which, since Shaffer (35) and Bürger (10), the quotient of the total daily amount of creatinine in mg, divided by the body weight (in kg) for the definition of muscular mass is to be understood. Perhaps it would be better to use the relation of active cellular mass to the amount of creatinine excreted daily. Ryan (32) gave as coefficient for the muscle mass the equation:

$$\text{Muscle mass} = \frac{40 \text{ (creatinine coefficient)}}{21.6}$$

In the following, reference is made to a balance of 50 cases of malnutrition edema published as long ago as 1949 (4, 5) in which the nutritional status of a group of 50 returned prisoners of war was observed and published in the form of debit and credit. In accordance with the restricted technical methods which we had at our disposal, all tests mentioned above could not be made and especially the determination of the amount of fat was omitted which would have been very conclusive at that time. The men were rid of their edema and were then in the dry status of protein deficiency. It was found that these men, after several years of most severe malnutrition, most of them having been hospitalized with extensive edema and whom we had been able to feed, thanks to the help of the British Salvation Army, had lost between 29% and 57% of substance in comparison to an average computed in accordance with normal figures. In detail, above all, the total haemoglobin content—computed from the amount of blood in circulation, determined with Evan's blue and the percentile Hb content—showed a reduction of over 50%. The creatinine coefficient was reduced by −29%, although the muscles of these men, who had been burdened by heavy work until only a short time before their arrival at the clinic, were extraordinarily reduced by 42% of the amount of creatinine computed as normal. The subcutaneous fatty tissue was entirely missing. The reduction of basal metabolic rate was comparatively slight. It did not lose so much, because all stored fat which did not respire was used up. Unfortunately, at that time we were unable to determine the extracellular fluid which would have allowed an indirect measurement of the amount of fat. This research was undertaken in 1947 to 1949.

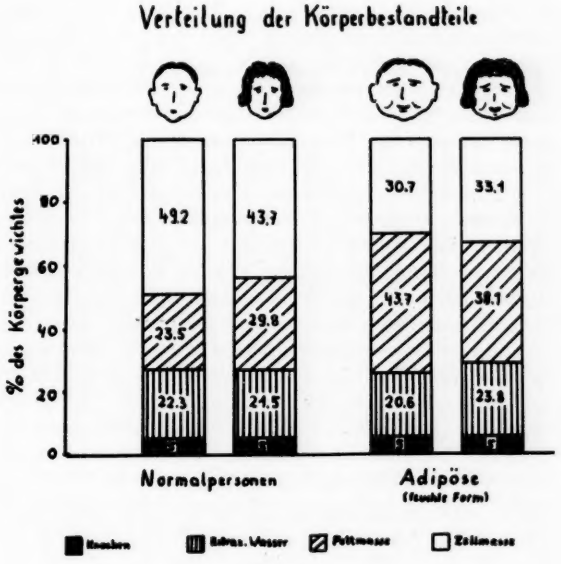

FIG. 4

The clinician will not very often have made use of such a survey, as he usually has to bring the bad nutritional status of his patients into connection with the primary illness. Nevertheless, severe conditions of deficiency are to be expected in much chronic absorption damage on the part of the alimentary tract, as well as in many hormonal disturbances and in carcinoma.

For ordinary clinical work, the serum protein analysis and the determination of Hb are in general sufficient, whereby the serum cholesterin and perhaps total fat can be added. In the conditions which accompany overweight, such as obesity, it is the abnormal amounts of fat which are of interest. In figure 4 normal figures are compared with those in obesity. This is not the place to discuss clinical characteristics; the intention was only to show the enormous increase in fat tissue in the obese.

Now we turn to the dynamics of the protein status, which is the protein metabolism in the wider sense. The protein balance in the healthy person is amazingly well adjusted. It was one of the basic principles of older nutritional research that an increase in protein reserves of a healthy person is not possible. Certain reserves similar to protein pools were recognized, of course, and that these reserves can be easily filled up after preceding undernutrition in the form of slow starvation. But it was a most astonishing discovery that the slightest interference in the metabolic balance of the organism, such as a minor operation or even the immobilization of an extremity, can lead to a negativity of the N-balance. In Germany, it was Bürger and Grauhan (11) who as long ago as 1923 pointed out that in major operations—independent of the appearance of fever—an increase in the excretion of nitrogen resulted. Since then, in extended research, particularly by Elman (16, 17), Wiley (38) and many others in the U. S. A. and Magee (27)

in Great Britain, these problems have been studied and the catabolism of the protein stores is a very important characteristic behaviour of the metabolism of a sick person. Even the complete immobilization of the lower extremities in plaster, as Whedon and co-workers (37) have shown at the New York Cornell University, leads to a considerable disequilibrium of the total metabolism. The authors, by constructing a swinging bed, managed to correct the considerable N-losses which had occurred through the complete immobilizing of persons who were otherwise completely healthy. This is no doubt caused in particular by muscle wasting, while through the rocking bed slight muscular contraction took place.

The method of determining the nitrogen balance has become the object of a very large number of discussions and it would be a good thing to indicate, for clinics especially, the difficulties of their exact determination. Nevertheless, I am of the opinion that exaggerated accuracy is not necessary for the clinician with his much wider range of problems. In the case of the seriously ill, with their lack of appetite and the difficulty of measuring all excretions exactly, detailed exactness is not so important if only the general direction of the metabolism is recognized. Thus, during the war, we measured not only the urine but also the amount of pus of the wounded and computed the nitrogen loss.

The nitrogen loss in empyema and purulent infections can be considerable. The N-loss in the faeces, on the other hand, is usually very stable and only becomes higher in malabsorption syndrome, especially in stomach resection with greatly accelerated passage through the resected stomach, in pancreatitis and pancreas carcinoma, in bile excretion trouble, in Whipple's disease, diarrhoea, sprue, and in severe colitis. Only in such cases must a higher N-loss than usual (1 — 1.5 g N) be expected of the N-excretion through the intestine, and it must be determined carefully. There is also malabsorption in diarrhoea resulting from protein deficiency edema and this can increase to quite high N-loss. Finally, secretory processes in the intestinal lumen must sometimes be taken into consideration. Naturally, the urine must be carefully collected and ammoniacal decomposition avoided through addition of acids. Much more difficulty usually arises from the intake side. The control necessary in clinical departments schooled in dietetics, with careful computation of the caloric and protein composition of the diet, does seem, however, to be sufficient to find the balance, even if the protein content of a dish really should be controlled by a collective analysis of the meals, if the utmost accuracy in the balance were concerned. But this will certainly seldom be the case in the formulation of clinical questions. Thus, in my opinion, enough has been said about the methodic part of finding the 'clinical evaluation of the calorie and protein status.' Refinements and improvements will best arise from the discussion of these methods and their individual clinical indications and application.

I hope that I have not left important problems untouched. After all every status, with continuous controls, will show a dynamic course of events, as was seen at the conclusion of a progress control which showed that a severely dystrophic patient had recovered within 85 days to practically normal. A young organism, if the actual cause of his protein deficiency and his malnutrition have been removed, for instance after controlling the infection in a case of severe wounds with empyema, is able to make good his losses in an astonishingly short time. There is a temporary phase with a great deal of fat formation, as I described in the case of deficiency patients with lipophile dystrophy and as also can be seen from the research on volunteers by Keys (23).

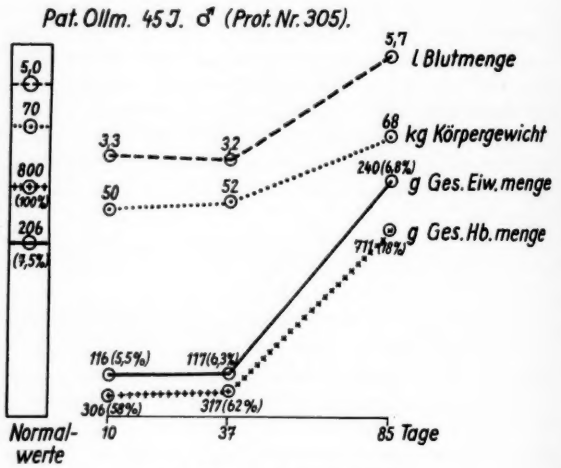

FIG. 5. Recovery from starvation.

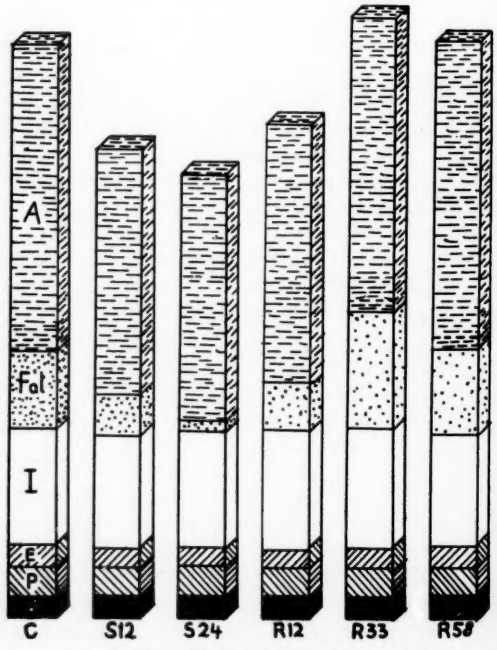

FIG. 6. (From Keys and coworkers, no. 23).

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Clinical evaluation of vitamin and mineral status in man

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THE SUBJECT of evaluation of nutritional status is of great importance, especially in the underdeveloped countries, in view of the increasing consciousness on the part of all concerned in improving the living standards and the nutritional levels in population groups. I have been interested in this subject for over two decades, as an experimental pathologist, clinician and public health nutritionist. Further, I have had the privilege of being associated with the World Health Organization and this has given me more opportunities to visit countries in different parts of the world and study some of the nutritional problems. With this background, I propose to present to you some of my observations in this field with the main object of eliciting your views and sharing your experiences.

All of us are quite familiar with the typical signs and symptoms of vitamin and mineral deficiencies. During the course of nutrition surveys on different population groups some specific signs and symptoms have been described, associated with malnutrition. The exact significance of these findings and their relationship to known nutrients are, however, not very clear. During the discussion that follows, I wish to draw your attention to some of these entities. For the sake of discussion, the clinical signs are described in relation to the various sites rather than to their etiological factors.

Nutritionists in India have paid adequate attention to the study of nutriture or the evaluation of nutritional status in man. Under the auspices of the Indian Council of Medical Research, leading workers in this field have formulated specific schedules (see Appendixes A–E). In a country where there is overt malnutrition and large population groups have to be covered in a short time, it was considered necessary to evolve a schedule which would permit a rapid clinical evaluation. It will be seen from the schedule appended that apart from a general examination, attention is paid mainly to a study of the eyes, skin and mouth.

OCULAR MANIFESTATIONS

Xerophthalmia and keratomalacia. The ocular manifestations in vitamin A deficiency are well known. The cornea and conjunctivae become dry—xerophthalmia. The conjunctivae may be thickened and wrinkled. In some cases, chalky white foamy spots—Bitot's spots—may be observed at the limbus conjunctivae. In severe cases the process of xerosis may extend to the cornea which becomes opaque, soft and necrotic—keratomalacia. If left untreated, the cornea may perforate, resulting in total blindness.

Some workers make a distinction between xerophthalmia and keratomalacia. Are these different conditions or is keratomalacia a severe form of xerophthalmia?

The question may perhaps be best answered by the results of experimental vitamin A deficiency. I have studied in great detail the macroscopic and the histological changes in rats and rabbits fed on experimental diets, low in carotene and vitamin A (2). The earliest lesion in the eye is one of xerosis. Histologically, the superficial layers of the epithelium lining the cornea and the conjunctivae, especially near the limbus, show marked keratinization. As the deficiency advances, the cornea becomes opaque, soft and necrotic, similar to that seen in human cases. Perforation of the cornea and prolapse of the iris are also seen in severe cases.

Keratomalacia is commonly seen in undernourished children and has been reported as one of the commonest causes of preventable blindness in India. I have seen cases where the cornea melts away, as it were, in a short time. As the condition is often painless, much damage is done to the cornea before the child is brought in for treatment. It is possible that in an undernourished child showing manifestations of vitamin A deficiency, keratomalacia may be precipitated by infections or infestations. I have seen children in displaced persons' colonies developing keratomalacia after a severe bout of diarrhoea.

My experience has been, based on clinical and experimental evidence, that both xerophthalmia and keratomalacia are due to the same pathological process, the difference being only in degree.

Night blindness. Let me mention a few words about night blindness. It is generally recognised that one of the earliest manifestations of vitamin A deficiency is night blindness. In a population which is illiterate, this symptom is not easy to elicit. However, we have seen

cases where the mother has volunteered the statement that her child was unable to see properly in dim light. A leading question in older children has often elicited conflicting answers. Our experience has been that adequate emphasis cannot be put on this symptom in assessing the clinical nutritional status in illiterate population groups.

Large-scale incidence of night blindness in community groups has been rare in my experience. However, in one colony of displaced persons, we had a very high incidence of night blindness in children, at the same time, before thay were rehabilitated.

Punctate keratitis. Circumcorneal injection and corneal vascularity are often found in riboflavin deficiency. In addition, superficial punctate keratitis is also seen in a few cases. The latter is, however, not a common finding. I have seen only a few cases in South India.

Angular conjunctivitis. Angular conjunctivitis is known to be the result of infections. I have seen some cases where appearances similar to angular stomatitis were also seen at other muco-cutaneous junctions, e.g. around the eyelids, at the anal margin and around the vulva. Is this a manifestation of riboflavin deficiency?

Pigmentation of conjunctiva. Various degrees of pigmentation of the bulbar conjunctivae are sometimes met with in undernourished children. The pigmentation may be diffuse or patchy. In some instances, the pigmentation is seen as fine spots or dots at the end of blood vessels. The nutritional significance of these signs is doubtful. It is probably not a sign of vitamin A deficiency.

CUTANEOUS MANIFESTATIONS

While the skin is affected in malnutrition, unfortunately there has not been much clarity with regard to the nomenclature and its description. In vitamin A deficiency, the skin may become dry. In the exposed areas, specially on the legs, the skin may present the appearance of 'fish skin' also known as ichthyosis. I have seen in some cases with marked keratomalacia, the skin presenting the appearance of a 'giraffe skin', the scales from the dry skin falling off easily.

In addition, the skin may appear dry and rough. Papular eruptions commonly known as phrynoderma (follicular hyperkeratosis) may be present at the sites of the hair follicles. The lesions—dry, firm, spiny papules of the size of a pinhead—begin to appear first over the anterolateral surface of the thighs and the posterolateral surface of the upper arms.

While there has been some unanimity regarding the etiological relationship between vitamin A and dry skin, there has been some controversy regarding the exact nutritional significance of the follicular dermatosis.

My earlier work (3, 5) based on clinical observations, examination of biopsy material and therapeutic trials has shown that phrynoderma is probably a "manifestation of a nutritional deficiency in which lack of vitamin A is an important factor." Subsequent studies by workers in India and elsewhere have shown that deficiency of essential fatty acids may also play an important role in the production of this follicular hyperkeratosis. This aspect of the problem deserves discussion at this Congress, in some detail.

In order to elucidate this problem, we have carried out experimental work in the albino rat (1). Experiments were designed to study the effects on the eyes and skin of synthetic diets containing 50, 5, 1, and 0 IU of vitamin A. Similarly, experimental rats were fed diets containing 5% peanut oil, 5% lard, 5% hydrogenated oil and a fat-free diet.

In rats fed a vitamin A-deficient diet, there was superficial hyperkeratosis of the epidermis with consequent plugging and dilatation of hair follicles with slight atrophy of their lining epithelium, which is homologous to those occurring in cases of phrynoderma and keratomalacia.

In rats maintained on diets low in essential fatty acids, similar but less marked changes were noticed. One marked difference, however, was the absence of atrophic changes in the lining epithelium of the hair follicles and sebaceous glands, constantly seen in vitamin A-deficient animals. It may be noted that in phrynoderma the lining epithelium of the hair follicles and sebaceous glands show atrophic changes, similar to those seen in the experimental animals. This lends support to my earlier observation, namely that phrynoderma is probably a "manifestation of a nutritional deficiency in which lack of vitamin A is an important factor."

The next point which we may discuss with some profit is whether folliculosis and follicular hyperkeratosis are one and the same condition or are they different entities. My own impression, based again on histological examination of biopsy material from cases of keratomalacia (4) and phrynoderma, is that both folliculosis and follicular hyperkeratosis are stages of the same pathological lesion. Even in cases where the skin is only dry but without any papular manifestations, histological examination shows widening of the hair follicles, marked keratinization of the lining epithelium and accumulation of keratotic material; only keratotic plugs are not seen.

In view of the fact that much attention is paid to the skin lesions in the clinical assessment of nutritional status, a contribution from this Congress will greatly help nutritionists in their field studies.

'SORE MOUTH'

Under this head are included cheilosis, angular stomatitis and glossitis in various stages.

In clinical practice and field surveys, it is often difficult for us to differentiate between subacute riboflavin and niacin deficiencies, especially in the absence of other concomitant signs and symptoms, specific of these deficiencies. Is it possible to differentiate riboflavin deficiency from niacin deficiency, in the absence of other concomitant symptoms? What is the relative

etiological relationship of vitamin B_{12} and folic acid, as far as tongue lesions are concerned?

I may perhaps here digress for a minute to relate to you my experiences as a World Health Organization consultant in one of the countries in the Far East. During the course of an extensive country-wide nutrition survey, I saw a large number of cases of 'sore mouth', especially in pregnant and nursing women. Their principal diet consisted largely of polished rice; fish was consumed in small quantity; milk and milk products were rarely consumed. None of the other signs and symptoms of either riboflavin deficiency or niacin deficiency were clinically noticed. Is this a mixed deficiency of riboflavin and niacin?

MINERAL DEFICIENCY

Iron deficiency. While it is generally admitted that clinically secondary anaemia is common, the several causative factors are difficult to determine in a topical country where several infections and infestations are commonly present. In computing the dietary intake from data collected from diet surveys, an appreciable amount of iron is apparently present in the diet. Nutrition surveys have, however, revealed the incidence of anaemia.

The point which arises is: *a*) how much of the iron present in the diet is biologically available and *b*) how far is protein deficiency responsible for the anaemia?

Calcium deficiency. Even in a city like Bombay, where the nutritional levels are higher than in many rural areas, cases of infantile rickets are seen. These are confirmed radiologically and by biochemical studies. Enlargement of epiphyseal ends, especially at the wrists and 'beading of the ribs,' is the clinical sign used by the pediatrician and the nutritionist in the clinical diagnosis.

Apart from these findings, stigmata of healed rickets are usually recorded during the clinical examination of school children. Is this necessary? Has it any bearing on the nutritional status at the time of the examination?

Before I conclude, I propose to raise for discussion a point of general interest. Is it feasible to assess a quantitatively nutritional status from a score given to the various clinical signs and symptoms? My impression has been that this cannot be achieved in actual practice, as an equal weightage cannot be given to the several signs and symptoms included in the nutritional assessment schedule.

Limitation of time does not permit me to speak further on the various aspects mentioned above. For the same reason, I have not attempted either to review the literature on the subject or include a complete bibliography. I, however, feel that the points raised above for discussion are of fundamental importance in our studies on the clinical evaluation of vitamin and mineral status in man.

APPENDIX A

Schedule for Rapid Nutritional Survey

Serial No._____
Name_____
Age_____
Sex_____
Place_____
Weight (lb.)_____
Height (in.)_____
Haemoglobin (g/100 ml)_____

GRADE I

GRADE II

a. Poor musculature
b. Deficient subcutaneous fat
c. Mild anaemia
d. Lack of interest in surroundings
e. Mild signs of not more than one of the specific nutritional disorders or deficiencies mentioned under Group III of Grade III

GRADE III

GROUP I. Equal weightage—highly significant of malnutrition.
Nutritional oedema (dependent or generalized)
Gross muscular wasting
Marked anaemia
Xerosis of cornea

GROUP II. Significant of malnutrition
Tenderness of the calf
Red and/or raw tongue
Glazed tongue
Stomatitis
Bleeding gums
Angular conjunctivitis (bilateral)

GROUP III. Less significant
Xerosis or pigmentation of conjunctivae
Bitot's spots
Caries
Dry and/or rough skin
Crazy pavement skin
Hyperkeratosis

Special remarks if any:

APPENDIX B

Nutritional Assessment Schedule

No._____ Date_____
Village_____ District_____
Name of institution/family_____
Name of subject_____ Occupation_____
Examiner's name_____

			Remarks
		1. Sex	
		2. Age	
		3. Height	
		4. Weight	
		5. Hip width (intercristal)	
I. GENERAL		6. Appearance	0 1 2 3
II. EYES			
	A. Conjunctivae	7. Xerosis	0 1 2 3
		8. Pigmentation	0 1 2 3
		9. Discharge	0 1 2 3
	B. Cornea	10. Xerosis	0 1 2 3
		11. Vascularisation	0 1 2

c. Lids	12. Excoriation	0 1 2
	13. Folliculosis	0 1 2 3
	14. Angular conjunctivitis	0 1
d. Functional	15. Night blindness	0 1
III. MOUTH		
a. Lips	16. Condition	0 1 2
b. Tongue	17. Colour	0 1 2 3
	18. Surface	0 1 2 3
c. Buccal Mucosa	19. Condition	0 1
d. Gums	20. Condition	0 1 2 3
e. Teeth	21. Fluorosis	0 1 2 3
	22. Caries	0 1 2
IV. HAIR	23. Condition	0 1 2 3
V. SKIN		
a. General	24. Appearance	0 1 2 3
	25. Elasticity	0 1 2
b. Regional	26. Trunk	0 1
	27. Face	0 1 2
	28. Perineum	0 1
	29. Extremities	0 1
VI. ADIPOSE TISSUE	30. Quantity	0 1
VII. OEDEMA	31. Distribution	0 1 2 3
VIII. BONES	32. Condition	0 1
IX. HEART	33. Size	0 1 2
X. ALIMENTARY SYSTEM	34. Appetite	0 1
	35. Stools	0 1
	36. Liver	0 1
	37. Spleen	0 1
XI. NERVOUS SYSTEM	38. Calf tenderness	0 1
	39. Paresthesia	0 1

Signature of Examining Officer

KEY TO NUTRITIONAL ASSESSMENT SCHEDULE

1. Sex
2. Age
3. Height*
4. Weight†
5. Hip width (intercristal)

I. GENERAL

6. Appearance
   - 0 Good
   - 1 Fair
   - 2 Poor
   - 3 Very poor

II. EYES

a. Conjunctivae

7. Xerosis
   - 0 Absent, glistening and moist
   - 1 Slightly dry on exposure for ½ minute, lack of lustre
   - 2 Conjunctivae dry and wrinkled
   - 3 Conjunctivae very dry and Bitot's spots present

8. Pigmentation
   - 0 Normal colour
   - 1 Slight discolouration
   - 2 Moderate browning in patches
   - 3 Severe earthy discolouration
9. Discharge
   - 0 Absent
   - 1 Watery, excessive lachrymation
   - 2 Mucopurulent
   - 3 Purulent

b. Cornea

10. Xerosis
    - 0 Absent
    - 1 Slight dryness and diminished sensibility
    - 2 Haziness and diminished transparency
    - 3 Ulceration
11. Vascularisation
    - 0 Absent
    - 1 Circumcorneal injection of blood vessels
    - 2 Vascularisation of cornea

c. Lids

12. Excoriation
    - 0 Absent
    - 1 Slight excoriation
    - 2 Blepharitis
13. Folliculosis
    - 0 Absent
    - 1 A few granules
    - 2 Lids covered with extensive granules
    - 3 Hypertrophy
14. Angular conjunctivitis
    - 0 Absent
    - 1 Present

d. Functional

15. Night blindness
    - 0 Absent
    - 1 Present

    N.B. Exclude other eye diseases not associated with nutritional defects

III. MOUTH

a. Lips

16. Condition
    - 0 Normal
    - 1 Angular stomatitis, mild
    - 2 Angular stomatitis, marked

b. Tongue

17 Colour
    - 0 Normal
    - 1 Pale but not coated
    - 2 Red
    - 3 Red and raw
18. Surface
    - 0 Normal
    - 1 Fissured
    - 2 Ulcered
    - 3 Glazed and atrophic

c. Buccal mucosa

19. Condition
    - 0 Normal
    - 1 Stomatitis

d. Gums

20. Condition
    - 0 Normal
    - 1 Bleeding and/or gingivitis
    - 2 Pyorrhoea
    - 3 Retracted

e. Teeth

21. Fluorosis
    - 0 Absent
    - 1 Chalky teeth
    - 2 Pitting of teeth
    - 3 Mottled and discoloured teeth
22. Caries
    - 0 Absent
    - 1 Slight
    - 2 Marked

IV. HAIR

23. Condition
    - 0 Normal
    - 1 Loss of lustre
    - 2 Discoloured and dry
    - 3 Sparse and brittle

V. SKIN

a. General

24. Appearance
    - 0 Normal
    - 1 Loss of lustre
    - 2 Dry and rough or crazy pavements
    - 3 Hyperkeratosis, phrynoderma

*Height is taken with the child standing heels together, and the occiput back, buttocks and heels touching the scales. The individual looks straight and is encouraged to take a deep breath and make himself as tall as possible without raising the heels. Then the height is recorded with a wedge-shaped block.

†Weight is preferably taken with standard clothing. For comparative purposes in longitudinal studies, weights are better taken always at a definite time in the course of the day, preferably before breakfast.

	25. Elasticity 0 Normal 1 Diminished 2 Wrinkled skin
B. Regional	26. Trunk 0 Normal 1 Collar-like pigmentation and dermatitis around the neck
	27. Face 0 Normal 1 Nasolabial seborrhoea 2 Symmetrical suborbital pigmentation
	28. Perineum 0 Normal 1 Scrotal or pudendal dermatitis
	29. Extremities 0 Normal 1 Symmetrical dermatitis with pigmentation, of glove or stocking type
VI. ADIPOSE TISSUE (to be judged by examination of the arm over the biceps)	30. Quantity 0 Normal 1 Deficient
VII. OEDEMA	31. Distribution 0 Absent 1 Oedema on dependent parts 2 Oedema on face and dependent parts 3 General anasarca
VIII. BONES	32. Condition 0 Normal 1 Stigmata of past rickets
IX. HEART	33. Size 0 Normal 1 Apex just outside nipple line 2 Enlarged
X. ALIMENTARY SYSTEM	34. Appetite 0 Normal 1 Anorexia
	35. Stools 0 Normal evacuation 1 Diarrhoea
	36. Liver 0 Not palpable 1 Palpable
	37. Spleen 0 Not palpable 1 Palpable
XI. NERVOUS SYSTEM	38. Calf tenderness 0 Absent 1 Present
	39. Paresthesia 0 Absent 1 Present

APPENDIX C

Department of Nutrition, Government of Bombay
Evaluation of Nutritional Status

A note in regard to additions and alterations in the Indian Council of Medical Research schedules for Rapid and Routine Clinical Nutrition Surveys.

A. RAPID SURVEY SCHEDULE

1. *Haemoglobin estimation.* This is not feasible when a 'rapid' survey of a large group of population is undertaken in the field. Moreover, genuine cases of anaemia can be detected by a closer examination of the skin, mucous membranes and nails, by any clinician. Hence, the estimation of haemoglobin may be omitted in this schedule.

2. *Dental caries.* Some children showed dental caries but, on a general examination, appeared to be healthy enough to be put in Grade I. In view of this observation on a large scale, 'Dental Caries' may be taken from Group III in Grade III.

3. *Pigmentation of conjunctivae.* The above is also true of pigmentation of conjunctivae; hence, it may also be deleted from its present position in Group III, Grade III.

4. *Angular conjunctivitis.* Angular conjunctivitis is also known to be the result of certain infections. The infection is occasionally present bilaterally. It is, therefore, suggested that the above signs (2, 3 and 4) may be taken out of the schedule but indicated as a footnote under the heading 'Signs with doubtful nutritional origin.'

5. *Grade II.* So often one observes an undernourished child who subjectively appears to be included in Grade II. But none of the specific signs (*a*, *b* or *c*) under Grade II are present. Is this permissible?

6. The item (*d*) under Grade II has no definite significance. It may be deleted.

7. Item (*e*) in Grade II may be omitted and replaced by the following in parentheses: Mild signs of not more than one of the specific nutritional disorders or deficiencies mentioned under various groups in Grade III.

8. Night blindness should be included as an item in Group II, Grade III.

9. Signs of early rickets are not included in the schedule. Observations such as delayed dentition, pigeon-shaped chest, enlarged wrists may be included. This item is particularly useful in examining infants and toddlers.

B. ROUTINE SURVEY SCHEDULE

1. In the absence of any column under conjunctivae, or the column for nails, it becomes almost impossible to indicate any score to an anaemic person.

2. Same is true of 'Deficient musculature.'

3. Some children scored 1 (one) against 'Appearance' (indicating 'fair' as opposed to 'good' health). The score against any other sign was nil. Is this permissible?

4. Haemoglobin estimation (for anaemic persons) may be included in the schedule.

5. The present score 0, 1, 2, 3 for the various signs and symptoms may be omitted, as it is not possible to quantitatively assess the state of nutrition by the simple addition of the scores. Instead, it is suggested that the presence or absence of the various signs and symptoms may be indicated as follows:

++ = Marked + = Present 0 = Absent

C. CHOICE OF METHOD

Provided the clinical nutrition survey is carried out by an experienced medical nutritionist, the 'Routine Method' is not considered necessary. In practice, it is extremely time-consuming. The 'Rapid Method,' on the other hand, is a more practical method and is useful for assessing the nutritional status of large population groups and in instituting feeding programmes.

On the other hand, the 'Routine Method' is more reliable in the hands of a beginner. It is useful in assessing the results of human feeding experiments, as it provides detailed data in the interpretation of results. Where gross malnutrition or undernutrition is not present, this appears to be the method of choice.

APPENDIX D

Schedule for Rapid Clinical Nutrition Survey (Revised)

Serial No._____ Date_____
Name_____ Sex (M/F)_____
Age: Years____Months____ Place_____
Weight (lb.)____ Height (in.)____
School_____ Std._____ Div._____

GRADE I

GRADE II

a. Poor musculature
b. Deficient subcutaneous fat

c. Mild anaemia
(Mild signs of not more than one of the specific nutritional disorders or deficiencies mentioned under various groups in Grade III)

GRADE III

GROUP I. Equal weightage—highly significant of malnutrition
- *a*. Nutritional oedema (dependent parts or generalized)
- *b*. Gross muscular wasting
- *c*. Marked anaemia
- *d*. Xerosis of the cornea

GROUP II. Significant of malnutrition
- *a*. Tenderness of the calf
- *b*. Red and/or raw tongue
- *c*. Glazed and atrophic tongue
- *d*. Angular stomatitis (cheilosis)
- *e*. Bleeding gums
- *f*. Excoriation of eye lids
- *g*. Night blindness
- *h*. Early rickets (delayed dentition)

GROUP III. Less significant
- *a*. Xerosis of conjunctivae
- *b*. Bitot's spots
- *c*. Dry and/or rough skin
- *d*. Crazy pavement skin
- *e*. Hyperkeratosis
- *f*. Stigmata of past rickets (enlarged wrist, pigeon-shaped chest, etc.)

Signs of doubtful nutritional origin:
- i. Dental caries.
- ii. Discolouration and/or pigmentation of conjunctivae.

Special remarks, if any:

Signature of Examining Officer

APPENDIX E

Nutritional Assessment Schedule (Revised)

No._____ Date_____
Village_____ District_____
Name of institution/family_____
Name of subject_____ Occupation_____

Haemoglobin (for anaemic persons) g/100 ml					*Remarks*
	1. Sex (M/F)				
	2. Age (yr. months)				
	3. Height (in.)				
	4. Weight (lb.)				
I. GENERAL	5. Appearance	G	F	P	
II. EYES					
A. Conjunctivae	6. Colour	o	+	++	
	7. Xerosis	o	+	++	
	8. Discolouration	o	+	++	
	9. Discharge	o	+	++	
B. Cornea	10. Xerosis	o	+	++	
	11. Vascularisation	o	+	++	
C. Lids	12. Excoriation	o	+	++	
D. Functional	13. Night blindness	o	+		
III. MOUTH					
A. Lips	14. Condition	o	+	++	
B. Tongue	15. Colour	o	+	++	
	16. Surface	o	+	++	
C. Buccal mucosa	17. Condition	o	+	++	
D. Gums	18. Condition	o	+	++	
E. Teeth	19. Fluorosis	o	+	++	
	20. Caries	o	+	++	
IV. HAIR	21. Condition	o	+	++	
V. SKIN					
A. General	22. Appearance	o	+	++	
	23. Elasticity	o	+	++	
B. Regional	24. Trunk	o	+		
	25. Face	o	+	++	
	26. Perineum	o	+		
	27. Extremities	o	+		
VI. NAILS	28. Colour	o	+	++	
	29. Shape	o	+		
VII. ADIPOSE TISSUE	30. Quantity	o	+		
VIII. MUSCULAR TISSUE	31. Quantity	o	+		
IX. OEDEMA	32. Distribution	o	+	++	
X. BONES	33. Condition	o	+		
XI. HEART	34. Size	o	+	++	
XII. ALIMENTARY SYSTEM	35. Stools	o	+		
	36. Liver	o	+	++	
	37. Spleen	o	+	++	
XIII. NERVOUS SYSTEM	38. Calf tenderness	o	+		
	39. Paresthesia	o	+		

Special remarks, if any:

Signature of Examining Officer

KEY TO NUTRITIONAL ASSESSMENT SCHEDULE (REVISED)

1. Sex
2. Age
3. Height (in.)*
4. Weight (lb.)†

I. GENERAL
5. Appearance
Good (G)
Fair (F)
Poor (P)

II. EYES

A. Conjunctivae
6. Colour:
o Normal
\+ Slight pallor
++ Marked pallor
7. Xerosis:
o Absent, glistening and moist
\+ Conjunctivae dry and wrinkled
++ Conjunctivae very dry and Bitot's spots present
8. Discolouration:
o Normal colour
\+ Slight discolouration or pigmentation
++ Severe earthy discolouration or pigmentation
9. Discharge
o Absent
\+ Increased lachrymation
++ Excessive lachrymation

B. Cornea
10. Xerosis
o Absent
\+ Dryness and diminished sensibility
++ Ulceration
11. Vascularisation
o Absent
\+ Circumcorneal injection of blood vessels
++ Vascularisation of cornea

C. Lids
12. Excoriation
o Absent
\+ Slight excoriation
++ Blepharitis

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* Height is taken with the child standing, heels together, and the occiput, back, buttocks and heels touching the scales. The individual looks straight and is encouraged to take a deep breath and make himself as tall as possible without raising the heels. Then the height is recorded with a wedge-shaped block.

† Weight is preferably taken with standard clothing. For comparative purposes in longitudinal studies, weights are better taken always at a definite time in the course of the day, preferably before breakfast.

D. Functional
13. Night blindness
o Absent
+ Present

N.B. Exclude other eye diseases not associated with nutritional defects

III. MOUTH

A. Lips
14. Condition
o Normal
+ Cheilosis, mild
++ Cheilosis, marked

B. Tongue
15. Colour
o Normal
+ Red
++ Red and raw
16. Surface
o Normal
+ Fissured
++ Glazed and atrophic

C. Buccal mucosa
17. Condition
o Normal
+ Stomatitis, slight
++ Stomatitis, marked

D. Gums
18. Condition
o Normal
+ Spongy and bleeding gums
++ Severe gingivitis

E. Teeth
19. Fluorosis
o Absent
+ Chalky teeth
+ Mottled and discoloured teeth
20. Caries
o Absent
+ Slight
++ Marked

IV. HAIR
21. Condition
o Normal
+ Discoloured and dry
++ Sparse and brittle

V. SKIN

A. General
22. Appearance
o Normal
+ Dry and rough
+ Crazy pavements or phrynoderma
23. Elasticity
o Normal
+ Diminished
++ Wrinkled skin

B. Regional
24. Trunk
o Normal
+ Collar-like pigmentation and dermatitis around the neck
25. Face
o Normal
+ Nasolabial seborrhoea
++ Symmetrical suborbital pigmentation
26. Perineum
o Normal
+ Scrotal or pudendal dermatitis
27. Extremities
o Normal
+ Symmetrical dermatitis with pigmentation of 'glove or stocking'-type

VI. NAILS
28. Colour
o Normal
+ Slight pallor
++ Marked pallor
29. Shape
o Normal
+ Spoon-shaped

VII. ADIPOSE TISSUE (to be judged by the examination of the arm over the biceps)
30. Quantity
o Normal
+ Deficient

VIII. MUSCULAR TISSUE
31. Quantity
o Normal
+ Deficient

IX. OEDEMA
32. Distribution
o Absent
+ Oedema on dependent parts
++ General anasarca

X. BONES
33. Condition
o Normal
+ Stigmata of past rickets

XI. HEART
34. Size
o Normal
+ Apex just outside nipple line
++ Enlarged

XII. ALIMENTARY SYSTEM
35. Stools
o Normal evacuation
+ Diarrhoea
36. Liver
o Not palpable
+ Slight enlargement
++ Marked enlargement
37. Spleen
o Not palpable
+ Slight enlargement
++ Marked enlargement

XIII. NERVOUS SYSTEM
38. Calf tenderness
o Absent
+ Present
39 Paresthesia
o Absent
+ Present

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Biochemical evaluation of nutritional status in man[1]

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VARIATIONS IN QUANTITY and composition of the diet are reflected by changes in the concentration of chemical substances in tissues and body fluids, and/or by the appearance or disappearance of specific metabolites. The primary limitations of the biochemical approach to assess nutritional status in man are the degree to which these changes can be measured by existing techniques, and the extent to which the nutritional origin of these changes can be clearly separated from the influence of genetic, environmental, physiologic, pathologic and other factors.

The nutritional spectrum for any single nutrient varies from frank deficiency at one extreme to optimal nutrition in the middle and overnutrition at the other extreme (55). Research on methods of evaluation of nutritional status should be orientated towards the search for 'signposts' that would determine specific nutritional states within this spectrum. At present however very few of these 'signposts' are known. In order to fill the need for practical guidance in the evaluation of biochemical nutritional studies, it is necessary to use methods which give useful information although they do not delineate the entire spectrum.

Most of the biochemical tests now available can be divided into those which measure changes that directly reflect the 'supply of nutrient' and those which detect biochemical changes reflecting metabolic alterations consequent to nutritional effects, i.e. 'biochemical nutritional pathology.' An example of the first is the concentration of plasma ascorbic acid, which is not filling a metabolic function but is merely in transit from one tissue to another. Its lack might or might not indicate the status of intracellular ascorbic acid. Examples of 'biochemical nutritional pathology' are a lowering of the quantity of plasma albumin, or the accumulation of pyruvate in the blood. Plasma albumin, for instance, is not a nutrient, but an internally synthesized metabolite, and its deficiency indicates a metabolic impairment which prevents synthesis from balancing catabolism. In thiamine deficiency, an accumulation of pyruvate means much more than a relatively low level of blood thiamine. As pointed out by Sinclair (81), an individual with a value of 5 mcg of thiamine per 100 ml, shown by a single blood analysis, could not be classed as deficient; in longitudinal studies, however, it became evident that 5 mcg represented a deficiency state for that subject. Accumulation of pyruvate, on the other hand, indicates impairment in the oxidative decarboxylation and points to biochemical thiamine deficiency.

Signs of 'biochemical nutritional pathology' appear when the concentration of an essential nutrient in the tissues decreases to a point where there is interference with metabolism. They may arise from dietary deficiency of the substance, or be due to poor absorption, impaired transport and decreased utilization. The detection of these signs of actual biochemical pathology is of great value and contributes conclusive evidence of functional nutrient deficiency. The practical use of these biochemical measurements for evaluating nutritional status is limited however by homeostasis. It would be impossible to discuss here the variety and complexity of the factors involved, but their net effect is against such biochemical changes. When the capacity of the homeostatic mechanism is overwhelmed by a nutritional deficiency the result is biochemical disease.

Nutrient supplies may be the food, the body reserves, or both, but concerning cell nutrition this distinction is not of practical significance. Indices of 'nutrient supply' usually are measures of the essential dietary constituents as they appear in the body fluids. Their decrease may mean no more than a low intake for a variable period of time. They indicate relative adequacy of intake but not the existence, nature or magnitude of nutritional disease.

Many nutritional conditions considered important by the hospital clinician are not a major concern of public health nutrition workers, because they do not occur frequently. Only nutrient deficiencies known to be the result of inadequate dietary practices in existing population groups will be discussed. Vitamins E and B_6 are included, although of doubtful significance by this criterion. Vitamin B_{12} and biotin are examples of nutrients omitted because it is unlikely that deficiencies of them occur in a significant number of human subjects as the result of an inadequate diet.

PROTEINS

Biochemical measures of protein nutrition may refer to either the relative adequacy of the dietary intake or

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the extent of protein depletion. Bengoa *et al.* (10) stated as recently as 1959 that "in spite of the enormous advances in this field of nutritional biochemistry, there is no new test available which can be used in field surveys as an indicator of protein malnutrition."

For adult population groups consuming a mixed diet and maintained in nitrogen equilibrium, the daily excretion of urinary nitrogen is a good estimate of protein intake. This approach, recommended in 1932 at the International Conference in Berlin (70), was used by De Venanzi in Venezuela (29). He found that in the population group studied, the low excretion of nitrogen in the urine paralleled a low intake of protein in the diet. However, the method has two main objections: first, the extreme difficulty of obtaining accurately timed urine specimens under field conditions, and second, in children, who are not in nitrogen equilibrium, the relationship between nitrogen intake and nitrogen excretion will vary with the degree of nitrogen retention. This in turn will depend largely on the biological value of the dietary protein and the physiological state of the child. A recent report indicates that the ratio of total urine nitrogen to creatinine in periods as short as 4 hours correlates well with the level of dietary nitrogen (69).

There are numerous reports of somewhat elevated total plasma proteins in population groups with inadequate protein intakes. No common pattern of serum protein fractions has been found (6), but it is quite obvious that the simple measurement of serum or plasma total proteins will not detect early stages of protein deficiency. When the deficiency is severe, the marked drop in total protein concentration is mostly due to a lowering of the albumin fraction. As in kwashiorkor this drop serves only as a confirmation of a condition which is already clinically evident.

More labile plasma nitrogen compounds related to protein metabolism would appear to offer a better possibility. Individuals living on chronically suboptimal intakes of protein reduce their 'protein metabolic level' and attain nitrogen equilibrium in the case of adults, or maintain a positive nitrogen balance in growing children, but at a lower plane. The phenomenon is not accompanied by changes in fundamental protein moieties like plasma albumin, until the deficiency is very severe.

Platt measured the urinary excretion of urea of children (66) and lactating women (67) of different nutritional and socio-economic conditions and found the ratio of urea nitrogen to total nitrogen to be markedly lower in the groups with poorer nutrition. We have tested this ratio using a single fasting urine specimen and have confirmed Platt's results for children of both high and low socio-economic groups in Guatemala. This ratio reflects essentially the magnitude of urea excretion, since the amounts of other urinary nitrogenous compounds are not altered as significantly. Therefore, the ratio of urea to creatinine in fasting urine samples also bears a direct relationship to the protein metabolic level of the individual.

This approach seems attractive since it measures a more labile aspect of protein metabolism than the synthesis of components as fundamental as plasma albumin and enzymes. Further studies should be conducted as to the feasibility and reliability of the test in single fasting urine samples under field conditions. Since this test requires that no immediate intake of nitrogen be reflected in the urine, it is recommended that a fasting specimen be collected after emptying the bladder in the morning.

The fasting level of plasma nonprotein nitrogen (NPN), of which urea is the most important fraction, is also influenced by the amount of protein in the diet. If this is large, the NPN will be maintained at a higher level than if the subject is subsisting on a low protein diet (65). This situation of partial protein restriction is not to be confused with starvation, where the plasma levels of NPN tend to increase. De Venanzi (28) determined the concentration of plasma NPN in laborers from Caracas, Venezuela, and found an average value of 23.9 mg/100 ml which is lower than values given in the literature for normal subjects. He attributes this low value to the diets poor in protein consumed by the group studied.

The level of plasma amino acid nitrogen has also been suggested as an indicator of human protein nutrition status (57). When Albanese (2) investigated the relationship of plasma amino acid levels to the dietary protein intake and the nutritional status of an individual, he found good correlation between plasma amino acid levels and the percent weight deficit, as long as the weight deficit did not exceed 30%. As the protein stores of a depleted subject became filled, the fasting plasma amino acid nitrogen increased. Plasma amino acid levels thus appear to be a sensitive measure of protein nutriture.

The finding, that in severe protein depletion not only plasma albumin but also the activity of some plasma enzymes are much reduced, stimulated investigations to determine enzyme plasma levels in population groups with suboptimal protein intakes as judged by dietary and physical examination data. Under field conditions, however, even very different socio-economic groups of children have no differences in serum pseudocholinesterase activity (4). Direct measurement of liver protein and enzymes have also shown patterns of changes associated with severe protein malnutrition (103). However, the effect of milder degrees of deficiency is not known in humans, and liver biopsy obviously cannot be done routinely to detect mild degrees of malnutrition.

It is well known that the sacrifice of skeletal muscle during protein deprivation protects more essential tissues. This occurs early, under conditions of insufficient protein intake. Stearns (87) says that maintenance of skeletal musculature is compatible only with adequate protein intake. The indirect estimation of muscle mass from 24-hour urinary creatinine excretion data has been recommended. Decreases in muscle mass are proportionately larger and their estimation more sensitive than decreases in body weight and body height. The expression 'mg of creatinine in 24-hour per cm body height' is to be

preferred over that of 'per kg of body weight' since variations in fat depots do not affect the former. Since the collection of 24-hour urine specimens is very difficult in the field, the use of carefully timed shorter periods deserves further testing. Examples of studies in which this technique has been successfully employed are those of Stearns (87) in North American children 1–10 years, Standard *et al.* (86) in kwashiorkor, and Arroyave *et al.* (5) in Central American children of different socioeconomic levels.

VITAMIN A

Inadequate dietary vitamin A activity, either as preformed vitamin A in foods of animal origin or as active carotenoids in vegetable foods, results in tissue decreases of this vitamin. Since the liver is the main site of vitamin A storage, its concentration in liver tissue is a direct measurement of vitamin A reserves. In the living human subject this can be determined in liver biopsy specimens, but, as mentioned before, this complicated procedure is impractical for the evaluation of vitamin A nutriture in population groups.

The serum levels of carotene and vitamin A have been used extensively to corroborate the diagnosis of vitamin A deficiency (36). The levels of carotene directly reflect changes in dietary intake although they are not per se indicative of vitamin A nutrition. Subjects receiving only preformed vitamin A in their diets may have adequate vitamin A reserves with practically no carotene in their plasma. However, in populations living on a mixed diet, particularly diets of vegetable origin, a low carotene level is very informative. If accompanied by a low plasma vitamin A level it is conclusive evidence of inadequate vitamin A nutrition.

Vitamin A plasma levels do not necessarily reflect recent intake. Large stores of vitamin A in the liver in well-nourished human adults tend to maintain the plasma level for about 1 year (98), whereas the child requiring an extra amount of this vitamin for growth might exhaust the liver reserves in a shorter time. In infants this seems to occur in 3 to 4 months (16).

While a marked decrease in plasma or serum vitamin A levels is compatible with a diagnosis of vitamin A deficiency, one might encounter occasional febrile conditions, not related to nutrition, which depress the vitamin A plasma concentration (37). Furthermore, the extremely low levels of serum vitamin A found in children with kwashiorkor may be the result of an impairment in blood transport since they may rise upon the recovery of the plasma albumin even with no dietary vitamin A (3). Such severe protein malnutrition cases would be clinically detectable and there is no evidence that milder degrees of protein depletion have an effect.

The ICNND manual for nutrition surveys indicates that serum levels of vitamin A between 20–50 mcg are acceptable, 10–19 mcg are low and below 10 mcg clearly indicate deficiency in young adult men (49). However, the level at which deficiency of vitamin A begins to limit human growth is unknown. Vitamin A values per 100 ml of serum for pre-school age children from lower income families in Amatitlan, Guatemala, were 11 mcg for the first quartile, 17 mcg for the second, and 22 mcg for the third (9). These biochemical results coincided with critically low intakes, although not a single clear-cut clinical deficiency was encountered in this study.

There is no readily determined biochemical measurement of metabolic derangement due to vitamin A deficiency. Although the regeneration of rhodopsin is known to decrease in rate as a result of severe deficiency of this vitamin (1), its measurement is obviously impractical for survey purposes. Incomplete knowledge of the mechanism of action of vitamin A in metabolism has prevented further advances in biochemical methods for the detection of its deficiency. A new hypothesis for the action of vitamin A has been put forward recently by Dowling and Wald (31), The protein moiety opsin needs to be bound to vitamin A to preserve its integrity, and these authors suggest that this might be true for other protein moieties in the body, such as those of the epithelial cells. Since plasma vitamin A alcohol is bound to the albumin fraction, the decrease in this plasma protein observed in vitamin A deficiency in cattle (33) could be the result of an accelerated catabolism of the albumin moiety when insufficient protecting vitamin is bound to it. Obviously more research is needed to clarify this point, but if the hypothesis is correct it offers a new basis for devising biochemical tests for vitamin A deficiency at the metabolic level.

VITAMIN D

Biochemical changes characteristic of nutritional disease due to deficiency of this factor have been known for some time. Obviously the adequacy of vitamin D nutriture cannot be correlated with dietary intake since body synthesis replaces the dietary need for all or part of this vitamin when sufficient exposure to sunlight takes place. The most specific laboratory measurement for the confirmation of the clinical diagnosis of rickets is the alkaline phosphatase serum level. The activity of this enzyme rises early in the development of the deficiency and tends to be proportional to the severity of the disease. Decrease in the phosphorus concentration of the blood serum is also a constant finding (48, 52) and helpful in diagnosis. Calcium serum levels decrease inconsistently and are unreliable as the only index of rickets (52).

The problem of detecting subclinical deficits of vitamin D is more complicated. The alkaline phosphatase serum activity of groups of children is not a generally accepted criterion. Bessey (11) suggested its measurement as an index of possible value "in determining adequacy of antirachitic agents," and this has been included in many biochemical-nutritional surveys. In general, the results have not been interpretable, mainly because the relationship of the degree of vitamin D deficit to the level of this enzyme in serum has not been established for subclinical deficiency. Since the levels of serum alkaline phosphatase

activity decrease in children with severe protein deficiency (30, 75), a tendency to rise due to vitamin D deficiency might be masked when the two conditions coexist.

In adults, osteomalacia may occur as the result of a deficiency of vitamin D and is manifested biochemically by an increase in the serum alkaline phosphatase and a decrease in the concentration of serum calcium. The increase in alkaline phosphatase is not specific, since it increases in a number of other pathologic conditions (37).

VITAMIN E

In 1954 Bessey (11) commented on the probability that the determination of blood vitamin E would find wide application in evaluating vitamin E nutrition if it were established that man required this vitamin. Two years later, Goldsmith (34) stated that vitamin E had not come of age in human nutrition. Four more years have elapsed and, despite the continuous efforts of some investigators to demonstrate the nutritional importance of vitamin E in human nutrition, Dr. Goldsmith's statement is still applicable.

Vitamin E deficiency has been observed in isolated cases in both adults and children. Useful information on the biochemical alterations which are characteristic of vitamin E deficiency has been derived from the study of these cases. Woodruff reported a case of deficiency due to lack of intestinal absorption in a patient suffering from xanthomatous biliary cirrhosis (106). The serum tocopherol level was 0 and the patient had creatinuria and excreted a pentose compound in the urine. The hydrogen peroxide hemolysis of the red blood cells (RBC) was high. Administration of large doses of vitamin E for several months raised the serum levels to 0.35 mg/100 ml, the creatinuria and pentosuria disappeared and the susceptibility of the RBC to hemolysis decreased. The relationship between vitamin E nutrition and integrity of muscle mass is evidenced by the creatinuria as well as the presence of pentose in the urine; the latter was probably derived from excessive destruction of nucleic acid. At least two of these biochemical changes have been observed in the human subjects of the well-controlled experiments at Elgin State Hospital (44), i.e. increased susceptibility of RBC to hydrogen peroxide hemolysis and lowered vitamin E serum levels. There were no other abnormal findings even after more than 3 years of observation.

Newborn infants have lower levels of plasma tocopherol than adults, but these increase in the breast-fed child (91). Premature infants fed partially skimmed milk are perhaps the nearest to primary deficiency of vitamin E; their low plasma levels at birth decrease even further to values around 0.13 at 2 months, at which time a high RBC susceptibility to hydrogen peroxide hemolysis is found (62).

In the absence of additional clinical or pathologic evidence these alterations are only suggestive of deficiency. Aside from premature infants, evidence of vitamin E deficiency in children, as in adults, is associated only with pathologic conditions causing impaired absorption of fat, such as cystic fibrosis of the pancreas and biliary atresia (63). The very low vitamin E serum levels found in kwashiorkor (75, 92) are probably the result of either malabsorption or impaired transport rather than deficient intake.

It is concluded that the following biochemical observations assist in the diagnosis of vitamin E deficiency: *a*) low vitamin E serum or plasma levels, *b*) increased susceptibility of the RBC to hydrogen peroxide hemolysis, and *c*) excretion in urine of metabolytes indicative of increased muscle destruction, such as creatine and pentoses.

ASCORBIC ACID

The concentration of plasma ascorbic acid decreases rapidly with dietary deprivation of this factor. Crandon *et al.* (26) found that it reached a level of 0 mg/100 ml in about 40 days, but clinical scurvy was not evident until the 143rd day of deprivation. From these results, confirmed by other workers, two practical conclusions may be drawn: *1*) that the ascorbic acid concentration in the blood plasma reflects the recent past intake of the vitamin, and *2*) that its disappearance from the plasma is not diagnostic of scurvy although it is compatible with it. The urinary excretion of ascorbic acid in 24 hours gives an estimate of the prevailing dietary intake. The ratio of ascorbic acid to creatinine in a random single urine specimen, preferably fasting, gives information of similar value (55).

There is strong evidence that the concentration of ascorbic acid in white blood cells (WBC) is a good estimate of its concentration in other tissues and, thereby, of total body ascorbic acid. With a sufficiently high intake, complete tissue saturation is attained. In the adult this intake is about 100 mg daily with a corresponding WBC ascorbic acid content of 20–30 mg/100 ml. At intakes of zero the WBC ascorbic acid of a well-nourished adult decreases to nearly zero in 3 to 5 months (26, 99) and scorbutic lesions soon appear. According to Lowry (55), in the lower range of intake and tissue saturation, the WBC ascorbic acid level is an admirable index of nutritional status. The extent to which the determination of ascorbic acid in WBC may be considered practical depends on the laboratory facilities available, but the assay is more complicated than the measurement of serum ascorbic acid. It has been suggested that ascorbic acid serum levels be determined as a screening test.

Although not applicable to field work, a more direct estimation of tissue ascorbic acid deficit is accomplished by Lowry's method (55) of administering large amounts (500–2000 mg) in divided doses throughout the day to avoid flooding. With suitable correction for metabolic destruction of the administered ascorbic acid, the amount retained should equal the absolute deficit. Load tests have also been employed in which the amount of ascorbic acid excreted in a given time is measured after the ad-

ministration of a dose of the vitamin, either orally or parenterally. A review by Vilter (96) points out that these methods indicate only whether or not the tissues are saturated, but are poor indicators of the degree of unsaturation. "If the load test is conducted with an amount of ascorbic acid much smaller than the tissue deficit, there is no return, or if the dose is given rapidly there may be spillage due to momentary flooding of the blood stream" (55).

Cheraskin *et al.* have evaluated the intradermal test with dichlorophenolindophenol (23) as an indicator of the degree of tissue saturation of ascorbic acid. Although the method is not specific, owing to the large number of other reducing substances present in the skin, it shows some degree of correlation with ascorbic acid nutrition. This fact, plus its great convenience, favors its use as a screening test in nutrition surveys, but the method needs further standardization before it can be recommended for this purpose.

The demonstration of Sealock and Silverstein (78), that the scorbutic guinea pig excretes abnormal tyrosine metabolytes when the amino acid is given, was later extended to premature infants (54) and adults (73) with severe ascorbic acid deficiency. The work of Steel *et al.* (88) showed that human adults maintained for as long as 78 days on a diet containing 7 mg or less of ascorbic acid excreted urine with a higher reducing power, but no increase in tyrosil compounds was noticed.

RIBOFLAVIN

When Burch *et al.* (19) described a micro-fluorometric method for the estimation of free riboflavin, flavine adenine mononucleotide (FMN) and flavine adenine dinucleotide (FAD) in blood, interest was aroused in these measurements as indicators of riboflavin nutritional status.

Despite the large number of workers who have determined blood riboflavin in humans, only a few have reported the relative usefulness of the different forms in which this vitamin occurs. In 1952, Suvarnakich *et al.* (90) studied the variations of free riboflavin and FAD riboflavin in normal subjects and concluded that free riboflavin was too variable to serve as a useful criterion for riboflavin nutriture, and that FAD has more diagnostic value in detecting riboflavin deficiency in view of 'its greater stability.'

The work of Bessey *et al.* (12) is an example of a well-controlled study of the relation between riboflavin intake and blood riboflavin measurements. These authors maintained 10 men on a diet supplying only 0.55 mg of riboflavin/day for 16 months, and 6 more on intakes of 2.55 to 3.55 mg/day. The most consistent and significant difference was in the red blood cell riboflavin content, being 10.0 to 13.1 mcg/100 ml in the deficient group and 20.2 to 27.6 mcg in the supplemented group. All subjects on the restricted intake showed clinical manifestations of ariboflavinosis, 3 of them very severely. Although the plasma levels of the free FMN riboflavin decreased in the deficient group, the variability was very large due to some striking exceptions. The plasma FAD and the white blood cell riboflavin did not decrease significantly. In a second experiment the levels of RBC riboflavin of 8 men were determined periodically during 9 months on 0.5 mg/day of riboflavin intake as compared to 4 control groups with 1.6 and 2.4 mg of daily intake. The RBC riboflavin in the restricted group decreased significantly within 7 to 8 weeks. The authors' conclusion that the RBC riboflavin concentration 'is a reasonably sensitive and practical index' of riboflavin nutritional status is entirely justified. This work is important in the sense that it relates the biochemical findings in the blood to a definite state of deficiency. Many values, 'low,' 'normal' or 'high,' have been reported for groups of population, but their meaning in terms of individual deficiency of riboflavin has not been clearly defined because the point of reference has been the presence or absence of clinical signs which are, to a large extent, non-specific.

Significant attention has been given to the urinary excretion of riboflavin. The direct measurement of the 24-hour urinary excretion of riboflavin has been studied under controlled conditions in terms of daily intake and, in some instances, in relation to the appearance of clinical manifestations of deficiency. There is a general agreement in the figures given by several investigators for excretions corresponding to intakes ranging from 0.5 mg to around 1 mg daily. Within those limits of intake about 9 to 14% of the ingested riboflavin is excreted daily (36). Some disagreement may be explained by the different ways in which diet composition can affect the biosynthetic production of riboflavin by the intestinal flora, since the actual amount of the vitamin entering the circulatory system is the sum of the dietary riboflavin plus that synthesized in the digestive tract.

In well-controlled experiments in human adult subjects, Horwitt *et al.* (45) observed that variations in riboflavin intake from 0.55 mg to 1.1 mg/day produced only a slow proportional increase in urinary excretion. Raising the intake to 1.6 mg daily increased sharply and disproportionately the absolute amount, as well as the percentage of the dose excreted in the urine. Subjects receiving 1.6 mg/day excreted 4 times as much riboflavin as did those receiving 1.1 mg daily. According to these authors this result suggests that an adult male, under the conditions of the experiment (2,200 calories per day), requires between 1.1 and 1.6 mg/day. Williams *et al.* (104) arrived at practically the same conclusion.

Load tests have been extensively used for evaluating riboflavin intake and nutrition. Of the various techniques available, the 4-hour excretion following the parenteral administration of a 1-mg dose of the vitamin is promising (94) and deserves further study.

For field surveys, the determination of riboflavin urinary excretion in a single nontimed specimen, preferentially fasting, may be used. An excretion of 150 mcg/gm creatinine has been estimated by Lowry (55) as indicative of tissue saturation. The *Manual for Nutrition Surveys* of ICNND (49) has set excretions of 80–270

mcg/gm of creatinine as acceptable for young male adults. Lowry (55) states that expressing urinary excretion per gram of creatinine has the advantage of tending to correct for body size. This might be true within narrow limits but does not mean, for instance, that a child's riboflavin excretion calculated per gram of creatinine should approximate that of a mature man. In fact, the Newfoundland study (7) shows evidence of a much larger excretion of riboflavin per gram of creatinine in children than in adults.

The finding is not surprising since children need and usually consume more riboflavin in relation to their muscle mass, while creatinine excretion is proportional to it. Therefore, relationships between riboflavin excretion in the urine and adequacy of intake or deficiency in children must be established *de novo*. This is an open field for nutrition research.

THIAMINE

The thiamine concentration in the plasma, red blood cells and white blood cells of man can be determined at present by using very small quantities of material (18). The plasma levels in man are so low (0.5–1.0 mcg/100 ml plasma) that available methods are not sufficiently sensitive. The red blood cells contain higher levels (6–9 mcg/100 ml) but they do not seem to lose their thiamine even under thiamine restriction compatible with clinical beriberi (20).

The relationship between urinary excretion and level of intake has been more extensively studied for thiamine than for any other vitamin. The 24-hour output, the 1-hour excretion on fasting, and the random specimen thiamine content per gram of creatinine have proved useful. They help to estimate the relative level of intake, particularly when comparing populations or when evaluating the effectiveness of dietary supplementation with thiamine.

Since the requirement for thiamine is dependant on the carbohydrate content of the diet, an increase in the latter without a proportional increase in thiamine intake can aggravate thiamine deficiency despite a dietary improvement. This was recently evidenced in children of a poor orphanage in Guatemala City (INCAP, unpublished data), when a marked improvement of the protein, vitamins and caloric content of their diet resulted in significantly lower urinary thiamine excretion.

Sebrell (79) has reviewed the abundant evidence for an adult thiamine requirement of 0.23 mg/1000 calories as recommended by the NRC. Subjects consuming less of the vitamin excrete very little of it in the urine (36). Unglaub and Goldsmith (94) have stated that if the mean 24-hour excretion is low in a group of individuals, potential or actual thiamine deficiency may be suspected.

A variety of load tests been have applied in the hope that they might measure tissue deficit (94). Although these tests reflect the intake of thiamine they do not identify clinical deficiency. Among the tests which have been proposed, Melnick's parenteral administration of 350 mcg of the vitamin per square meter of body surface, with determination of the thiamine excretion in the subsequent 4 hours (58), offers good possibilities for standardization. Load tests are, however, quite impractical for field studies. Measurement of the excretion of pyrimidine compounds has also been suggested by Pollack *et al.* (68) and Mickelsen *et al.* (59), but practical methods have not been developed.

Thiamine offers one of the best illustrations of biochemical nutritional pathology due to its deficiency. Lack of thiamine interferes with the entrance of pyruvate into the tricarboxylic acid cycle and this keto-acid tends to accumulate in the body fluids. An elevation of pyruvic acid in the blood is observed in thiamine deficiency, but lacks consistency and specificity (47). Stotz and Bessey (89) stated that the ratio of lactate to pyruvate is sufficiently altered in thiamine deficiency to be of diagnostic value. Horwitt (47) found basal blood levels of these compounds of limited value for estimating mild degrees of this deficiency, and developed a test based on the effect of glucose and exercise on the lactic and pyruvic acid blood levels. The results are expressed by an equation which relates the blood glucose, lactic and pyruvic acids, 1 hour after a dose of 1.8 g of glucose/kg body weight, and 5 minutes after completion of a standardized exercise. This is called the 'Carbohydrate Index.' Derangement in carbohydrate metabolism due to thiamine deficiency gives a high carbohydrate index. Figures below 15 are found in non-deficient individuals. This interesting approach to the diagnosis of thiamine deficiency has the disadvantage of being too elaborate for widespread application in field surveys. For the latter, thiamine excretion per gram of creatinine in single random urine specimens is a practical biochemical test which aids in the evaluation of thiamine nutritional status.

NIACIN

This water soluble vitamin differs from the others of the B-complex because an amino acid, tryptophan, serves as its precursor in the body of some animals, including man. According to Horwitt *et al.* (46) approximately 60 mg of tryptophan are equivalent to 1 mg of niacin. Another characteristic of niacin is that it is not excreted in the urine but is metabolized to at least two methylated derivatives, N^1-Methylnicotinamide (N^1-Me) and the 6-pyridone of N^1-Methylnicotinamide (pyridone). Goldsmith believes that the pyridone is the most important end-product of niacin metabolism (40).

Values for the concentration of diphosphopyridine nucleotide (DPN) for whole blood, serum and red and white blood cells of well-nourished adults were given by Burch *et al.* (21) using their own micromethod; in general, these confirm previous results with other methods. Morley and Storvick (61) give fasting levels of oxidized pyridine nucleotides in blood, N^1-Me in serum and the urinary excretion of a number of niacin metabolytes in 4 women under controlled dietary intake, supplying 8.7 mg of niacin and 770 mg of tryptophan daily. These data

are useful as a reference point for biochemical studies in states of deficiency or dietary restriction.

Klein *et al.* (52) indicate that the concentration of nicotinic acid in the blood cells of human subjects suffering from pellagra, and of dogs with 'black tongue' was not lowered. Vivian and co-workers (100) studied women on controlled niacin and tryptophan intakes. The lowest intake was 2.5 mg of niacin and 30 mg of tryptophan daily. The tryptophan intake was raised stepwise, and each level maintained for 6 day-periods. On daily intakes of 2.5 mg of niacin and 30 to 190 mg of tryptophan, the blood pyridine nucleotides (PN) decreased from 20 to 40% below control period levels. Increases in blood PN started at 270 mg of tryptophan intake and reached the values of the control 'non restricted' period on an intake of 670 mg. It is apparent that more investigations, under controlled niacin and tryptophan intakes, are required to establish the extent to which the blood levels of niacin, or its derivatives, are useful for the appraisal of the niacin nutriture of humans.

The urinary output of N^1-Me and pyridone decreases when the intake of niacin is restricted (74), an effect which is more marked and rapid on the pyridone (40). Low urinary excretion of niacin metabolytes is not necessarily indicative of clinical pellagra. Excretion levels compatible with 'adequate body stores of niacin' have been suggested by the preliminary studies of Goldsmith *et al.* (39). Their results indicate that on a 'corn diet' supplying 200 mg daily of tryptophan, an intake of 8–10 mg of niacin insures body niacin stores in human adult subjects.

The need for continuing such investigations is obvious. Studies should not consider the niacin content but the 'niacin equivalent' (46) of the experimental diets. The availability of methyl-donor compounds and the efficiency of the methylating process influence the formation of urinary niacin metabolytes independently of the balance between intake and need. Relative niacin nutriture may be appraised in field surveys by measuring the urinary excretion of niacin metabolytes in 24 hours, in a single random specimen in relation to creatinine or, as suggested in the *Manual for Nutrition Surveys* of ICNND (49), in 6 hours in a post-absorptive state. This manual recommends the determination of N^1-Me only, because of the relative complication of the assay for pyridone. The fact that normal persons may occasionally show a very low urinary excretion of niacin metabolytes and that under certain pathologic conditions an excess is excreted (36) makes their measurement unsatisfactory for evaluation of niacin nutrition in individual subjects.

The effect of the ingestion of doses of niacinamide on the urinary output of N^1-Me and pyridone has been explored. The principle that a high retention of the dose means a state of tissue depletion must be accepted with some reserve. As previously mentioned, impairment of methylation would also alter the excretion of these 2 niacin derivatives. This has been pointed out by Ellinger and Coulson (32) and is particularly applicable to tests in which a large dose is administered.

A variety of different conditions have been proposed for niacin load tests. The oral administration of 500 mg of the amide, followed by quantitative estimation of the extra 'trigonelline' plus nicotinic acid bodies, was early suggested (17, 65). Better knowledge of niacin metabolism has since resulted in more refined procedures. Unglaub and Goldsmith (94) favor using a relatively small dose of 10 mg of niacinamide followed by at least a 12-hour urinary collection. Another method proposed by Goldsmith *et al.* (38) is an interesting research tool to discover more about the relationship between the degree of tissue depletion and the urinary levels of niacin metabolytes. In essence, they measured the 24-hour excretion of N^1-Me and pyridone under a controlled diet, supplying 10 mg of niacin and 1 gm of tryptophan. Low excretions were characteristic of deficient patients as compared with normal subjects. An additional 10 mg of niacin to the basal diet resulted in a larger increase in excretion during the following 24 hours in the normal subjects; if a 10 mg supplement was continued for several days, adequately nourished subjects rapidly attained a high plateau in niacin metabolyte excretion while deficient patients required more time to reach maximum excretion.

For field surveys, estimation of niacin metabolytes per gram of creatinine in random urine specimens is recommended, since it contributes information corroborative of suggestive dietary and clinical data.

VITAMIN B_6

The wide and uniform distribution of vitamin B_6 among food stuffs has been pointed out by Snell and Keevil (83). In their opinion, few foods can be considered really poor sources. This fact explains why vitamin B_6 deficiency is not found as a naturally occurring nutritional disease. Nevertheless, interest in this factor has been recently aroused by observations suggesting an increased demand during pregnancy, and by the accidental occurrence of a serious outbreak of vitamin B_6 deficiency in infants fed a newly modified milk preparation, proved *a posteriori*, to be deficient in the vitamin.

Although the requirements have not been definitely set, figures around 0.03 mg/kg body weight/day for adults (105) and between 0.01 and 0.02 mg/kg body weight/day for infants under 6 months are accepted as approximate (42). Experimental observations of vitamin B_6 in man have been carried out, either by the administration of diets deficient in the factor or by the addition of a pyridoxine analogue. Typical of such studies is that of Snyderman *et al.* (84) who observed 2 infants given a diet low in vitamin B_6 for periods of 76 and 130 days. The subjects were mentally defective and the diet was an attempted therapy. Three biochemical features were observed: *a*) disappearance of pyridoxic acid from the urine, *b*) marked reduction of urinary pyridoxine excretion, and *c*) loss of ability to convert tryptophan to niacin.

Observations of adults in whom vitamin B_6 deficiency has been induced, either by the administration of desoxy-

pyridoxine (61, 97) or by a deficient diet (41), lead to the conclusion that the urinary excretion of xanthurenic acid without a tryptophan load is not a reliable index of early deficiency. On the other hand, the work of Greenberg *et al.* (41) shows that in adult subjects, only about 2 weeks on a synthetic diet devoid of B_6 are sufficient to produce a ten-fold increase in the response of xanthurenic acid excretion in 24 hours to a 10 gm dose of DL-tryptophan. The authors consider this "one of the earliest detectable manifestations of pyridoxine deficiency."

Chiancone (24) has defined conditions for a tryptophan load test which can be used as a biochemical index of vitamin B_6 deficiency in an early stage. Tryptophan in a single dose of 100 mg/kg body weight is administered to the subject and the amount of xanthurenic acid excreted during the following 24-hour period, expressed as the percentage of the dose of tryptophan, is called the 'Xanthurenic Acid Index.' Indices not exceeding the value of one are regarded as normal (95). Whether this or other experimental conditions should be chosen is secondary to the necessity of adopting a standardized technique in order to obtain comparable results. It is important to investigate the excretion curve of xanthurenic in detail after the test dose of tryptophan in order to see if shorter collection periods would give information as useful as that obtained with the 24-hour collections. This question is of obvious practical importance.

The work of McGanity *et al.* (56) suggests that the changes in blood urea after a load dose of alanine might be related with vitamin B_6 nutritional status. An abnormal blood urea curve was found in patients with hyperemesis gravidarum after a dose of 30 gm of DL-alanine; the levels of urea rose reaching a maximum at the 6th hour, but remained elevated for 12 hours, while in normal pregnant and non-pregnant women they decreased to the pre-test levels in that time. The administration of pyridoxine to the hyperemesis gravidarum patients corrected the abnormal response to the test.

Vilter *et al.* (97) showed the same type of abnormal response in 2 of 3 subjects receiving desoxypyridoxine to induce pyridoxine deficiency. Abnormal tests coincided with the presence of clinical manifestations of deficiency. The patient who showed no clinical deficiency, although receiving the vitamin antagonist, responded normally to the test at all times. The test, therefore, seems to reflect well-advanced deficiency but, apparently, is of no value for detecting it at an early stage.

In a recent work, Wachstein *et al.* (102) measured the concentration of plasma pyridoxal phosphate in subjects at different time intervals up to 24 hours after an oral dose of 100 mg of pyridoxine hydrochloride and found a lower response in pregnant women during the last trimester of gestation than in normal men and non-pregnant women. This agrees with previous results suggestive of an altered vitamin B_6 metabolism in pregnancy (101) and also illustrates another test, the results of which might be interpreted as reflecting relative tissue saturation in a similar manner to the load tests for other water-soluble vitamins. Since only one sample of blood is necessary 24 hours after the pyridoxine dose, this test may have a practical advantage over the tryptophan load test which requires the collection of 24-hour urine specimens.

Recently, Babcock *et al.* (8) have shown that subclinical deficiency of vitamin B_6 in adults reduces the serum glutamic-oxalacetic transaminase. Measurement of the increase in this serum enzyme following administration of vitamin B_6, "provides an indication that the vitamin B_6 supply had been inadequate for optimum protein metabolism." The test is less sensitive than the xanthurenic acid excretion test after a tryptophan load.

Research is needed on laboratory methods to measure vitamin B_6 as a complex and determine its three biologically active forms, pyridoxine, pyridoxal and pyridoxamine (80, 82). More practical procedures, particularly in micro-scale, applied to blood and other biological materials would facilitate further studies of the nutritional biochemistry of this vitamin.

CALCIUM

Among the biochemical abnormalities which appear as manifestations of severe calcium deficiency is a decrease in the concentration of calcium in serum, which may reach values of less than 4 mEq/liter (35). When this occurs tetany and other clinical signs become obvious so that the biochemical index has only confirmatory diagnostic value. The maintenance of plasma calcium concentration is accomplished through a mechanism in which several factors, each more important than moderate variations in dietary intake, are operating (50). Investigation of the relationship between dietary inadequacy of calcium and its possible biochemical indexes is further hindered by the fact that the optimal intake of this mineral is unknown (43).

IRON

When iron is not available to the body in sufficient quantities, because of either inadequate intake or excessive loss, several types of biochemical alterations have been shown to occur. The best known manifestation of biochemial pathology is an impairment in the biosynthesis of hemoglobin. The clinical entity is, of course, iron deficiency anemia. In the laboratory this is easily detected by determining the concentration of hemoglobin in the whole blood, aided by the simple microscopical study of stained blood smears.

Anemia, however, is an advanced manifestation of lack of iron. In view of the great importance of iron in other fundamental biochemical systems, such as the cellular electron-transport chain, interest is beginning to develop in the study of possible alterations in the efficiency of this and other iron-dependent mechanisms. Evidence from animal studies (13) supported by clinical observations (15) led Beutler to suggest that some of these mechanisms might suffer even before hemoglobin levels become subnormal. Although no practical biochemical method for evaluation of iron deficiency based on these changes

yet exists, such studies are important contributions to the definition of 'adequacy of intake' and 'requirement' for this mineral in terms of biochemical pathology which occurs earlier than blood changes.

Early depletion of iron body reserves may be detected directly, by the estimation of hemosiderin in the bone marrow (72). The relative difficulty of obtaining bone marrow specimens under field conditions, however, makes this method an impractical approach to the evaluation of iron nutrition in population groups.

A lowering of the plasma iron levels is, in general, a manifestation of relative lack of available iron. Although in some specific instances this may be due to pathologic conditions such as infection, the occurrence of generalized reduced plasma iron levels is presumptive evidence of latent deficiency. It may be recommended that in surveys, tests of iron-binding capacity of plasma (71) be made in a selected subsample. Since increased binding capacity is a primary characteristic of uncomplicated iron deficiency (14, 26), it would confirm the nutritional origin of low iron values.

Hypoproteinemia, due to protein malnutrition (kwashiorkor in children), may disguise the signs of iron deficiency. There is a suggestion that this condition produces a decrease in the specific iron-binding B-globulin of plasma, and consequently a reduced iron-binding capacity is found simultaneously with low plasma iron levels (53). In these cases, however, the signs characteristic of a typical iron deficiency anemia appear when therapeutic diets low in iron are given (77).

IODINE

When insufficient iodine is available to the thyroid gland, this endocrine organ undergoes hyperplasia and hypertrophy, which in turn helps to maintain the supply of thyroid hormone of the body within limits compatible with normal metabolism. Decreased iodine availability to the gland may result directly from insufficient iodine intake or indirectly from the presence of goitrogenic factors, usually of food origin (25, 27, 93). The latter interfere with the normal uptake and utilization of iodine by an otherwise normal thyroid, although the actual mechanisms apparently vary with different compounds.

The 24-hour urinary excretion of iodine has been used to estimate the dietary intake. At least, the amounts excreted by persons in goitrous regions are markedly lower than those in nongoitrous areas (27). The work of Stanbury (85) clearly demonstrated an inverse correlation between the uptake of labeled iodine and urinary iodine excreted in euthyroid patients in the endemic area of Mendoza, Argentina.

Although the iodine bound to serum proteins reflects the functional state of the thyroid gland, its value as an indicator is very limited because goiter patients in endemic areas are generally euthyroid. It would be logical to expect that, in the initial stages of the development of goiter, an individual might develop a relative hypothyroidism before compensation occurred. This is suggested by the study of Scrimshaw *et al.* (76) who found that the relatively low-protein-bound iodine values found in a group of Central American children increased with the administration of potassium iodide or iodate simultaneously, with a decrease in the prevalence of goiter. To elucidate the value of this index more studies should be carried out of plasma protein-bound iodine in children living in endemic goiter areas.

The quantitative relationship between iodine intake and excretion and the presence of goiter is influenced by several physiologic, environmental and dietary factors. Research directed towards the study of this relationship should be conducted in different population groups with and without endemic goiter. Methods are needed to assess iodine nutrition at a subclinical level.

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Dietary determination of nutritional status

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DIETARY DETERMINATION OF NUTRITIONAL STATUS

IT IS COMMON GROUND (for example, 4, 11, 46, 49, 50–53) that nutritional status cannot be assessed by dietary means alone. The point was clearly made by Norris (46) who concluded: "If the results of a dietary survey show that the diet has a very low calorie content, the conclusion that the group in question is suffering from undernutrition is perhaps a legitimate one. But the fact that intake of certain nutrients falls below some recommended allowance does not justify the conclusion that a proportion of any group surveyed is suffering from malnutrition. In such circumstances the possible presence of malnutrition may be inferred, but the dietary survey *per se* provides no evidence of its existence."

What then are the nutritional reasons for collecting information on food consumption? Over 20 years ago Bigwood (4) gave a reason for conducting dietary surveys which is valid today. At its last session held in London in November 1937, the Technical Commission on Nutrition of the League of Nations Health Organization (26) had emphasized the value of making parallel studies of food consumption and of the nutritional state of the same human subjects. Referring to this recommendation Bigwood pointed to "the fact that such comprehensive surveys are rare at the present time, and yet offer the only means of enlarging our knowledge in regard to a physiological question of immediate topical interest—namely, the assessment of bodily requirements in respect of the vitamins and other protective principles." We still aim to use records of dietary intake of healthy individuals as a supplement to balance studies and growth and other records in the development of knowledge on the requirements of nutrients. We still seek to determine when and how health is likely to be impaired by a poor diet or improved by a better one. Earlier hopes of being able to find clear links between dietary intake and nutritional status have, however, been disappointed and we are increasingly aware that our problem is to find a means of assessing the dietary habits of a life-time—in the words of Thomson (52) "The nutritional status of a pregnant woman depends more upon her life-experience of diet than upon the nature of the diet she happens to take during pregnancy"—and to compare those with an individual's condition at the end of life (22).

As an example of the difficulties found in attempting to relate dietary intake to health, I should like to take the opportunity of drawing upon the findings of the Carnegie United Kingdom Dietary and Clinical Survey, the report (9) of which is not very readily available. This survey was made during the years 1937 and 1938 by a team of workers from the Rowett Research Institute, Aberdeen, and its objects, as given in the report, were *1*) to find out the kind of diet in families of different income groups in rural and urban districts in representative parts of the United Kingdom; *2*) to try to determine any correlation which might exist between diet and health; *3*) to give supplementary foods to a number of families most of whom were living on the poorest diets with comparable families as controls to determine the extent to which health and physique could be improved by improving diet.

The nutritive value of the diets was compared with standards devised in the Rowett Research Institute while the Survey was in progress and later with the recommendations of the British Medical Association (5). It was found that "In calories the average diet fell short of requirements in the lower expenditure groups; the associated shortage in total protein was more widespread and affected families further up the scale of expenditure; in calcium, vitamin A and vitamin C shortage was almost universal occurring in all but the groups of families in which expenditure on food was greatest." On the basis of these findings a feeding experiment was made at 5 places. The foods given varied in different centres and included milk, oranges, halibut liver oil capsules, marmite, cheese, eggs and nourishing soup, not all of which were given to all children taking part in the experiment. The children were examined clinically before the feeding experiment and after nearly a year of supplementary feeding with the following conclusion: "weight and height measurements of these children in comparison with similar data for children who had received no extra food showed that growth had been accelerated. The clinical examinations failed to detect a measurable difference between the groups with and without supplementary foods."

The lessons which have been learned since the Carnegie Survey was conceived may be summarised by saying that accurate information about dietary intake of populations, groups or individuals can form the basis of clinical or epidemiological investigation; it can help to confirm clinical diagnosis of deficiency; if collected over time on comparable groups, it can provide a basis

for long-term comparison of dietary and health records; it can suggest ways in which the diet can be improved by legislation or education. The more successful the attempts to determine the long-term effects of dietary intake on nutritional status the more fruitful will be the efforts of governments and others to improve the health of populations by legislation concerning the composition of foods, by supplementary feeding in schools and other places, and by programmes of education in nutrition and meal planning.

The main purpose of this review is to describe how records of food consumption are obtained. For this purpose, it is assumed that suitable means of converting records of foods consumed into nutrients and for comparing these with nutritional requirements are available, though the pitfalls met in both procedures are well recognized (see, for example, 20, 47, 62). In 1944, Sinclair (50) gave uncertainties on food composition and on human nutritional requirements as the main reasons why dietary surveys by themselves could not then 'provide evidence of the extent of malnutrition.' This is still true today.

There are two ways of obtaining information, indirect and direct.

INDIRECT ASSESSMENT OF FOOD CONSUMPTION

The indirect method is that described by the Technical Commission on Nutrition of the League of Nations Health Organisation (26) as a means of determining the total available quantities of foods from statistics of production, imports and exports. This method was classified as the 'First Type of Food Consumption Study.' It was developed during World War II and is now in use in many countries. It is described fully by FAO (15). In brief, the quantities of foods produced in a country are adjusted to allow for imports, exports and changes in stocks to give gross food supplies. From these are deducted the amounts of foods used for animal feed, seed and manufacture and the amount wasted during production and distribution up to and including the 'retail' level of consumption. The remainder represents the total food supplies available to go into the shopping basket, that is before they reach the kitchen.

Such estimates vary in reliability in different countries and even in the FAO publication of 'Food Balance Sheets' (e.g. 16) there is some inconsistency in the method of expressing, for example, fat supplies: some are expressed as unprocessed oils and some as processed fats. If such inconsistencies are not noticed they can lead to false comparisons and unnecessary calculations (see, for example, 27). These total supply estimates relate to food supplies for a country or territory as a whole: they can never provide information for groups of the population, for example, those living in different regions, belonging to different economic, social or occupational groups or of different ages. Notwithstanding their limitations, they give a useful indication of the general patterns of food consumption, especially trends in consumption over time. They can form a basis for establishing programmes of agricultural production, food processing and food education, and they can give an indication of the ways in which a national food supply might be nutritionally inadequate: total supply estimates were, for example, used to indicate ways of remedying national inadequacies of diet between the two medical surveys of nutrition in Newfoundland in 1944 and 1948 (31, 32), despite some difficulty in measuring precisely changes in supply between the 2 years. Such total supply estimates cannot make any direct contribution to the determination of nutritional status.

DIRECT ASSESSMENT OF FOOD CONSUMPTION

There are many variations of the direct method of obtaining information on food consumption. The success of any method depends to a great extent on the training, integrity, attention to detail and enthusiasm of the investigators. The Technical Commission on Nutrition (26) classified them into 3 types—Community or Institution; Family; Individual—and this classification still obtains.

The community or institution type, by which was meant studies on groups of the armed forces or in institutions with a large number of residents, such as boarding schools, hostels, prisons or religious communities, present fewer problems than the other 2 and will not be considered further in this review. In passing it is, however, of interest to quote Groen's (25) ingenious use of 2 communities of monks, Trappist and Benedictine, of different dietary habits but similar modes of life as a means of studying the effects of diet on serum lipids and on the incidence of atherosclerosis and coronary occlusion.

Family enquiries. Studies of the food consumption of families have been made frequently and in many countries. The informant is usually the housewife and the information required is a record of the food taken into the kitchen for a specified period of time to feed all members of the household. The information sought must be within the knowledge of the housewife, and it will be difficult to obtain records of foods and drinks taken in catering establishments, and of such commodities as ice cream, chocolates and soft drinks, which may be bought on impulse by members of the family. It is not easy in such studies to obtain information on distribution within the family, although in at least 4 studies (8, 29, 63, 64) this appears to have been achieved successfully. Nor is it easy to obtain records of the amount of edible food wasted. If dietary data are to be used to help in the assessment of nutritional status of individuals such information is, however, essential.

Before discussing some of the techniques that have been used and some of the results obtained, it may be useful to recall the views of those who attended the FAO Nutrition Meeting for Europe in June 1958. The purpose of the meeting was to bring together a group of European workers to review nutrition problems of importance in Europe, and one of the two major topics discussed was

the study of food consumption, with special reference to fat. The group pointed out (19) that whereas data from food balance sheets are available for almost all European countries on an annual basis, the number of direct food consumption surveys made in Europe is comparatively limited and that "such surveys may not cover a representative sample of the population and may not be carried out in such a way as to permit a satisfactory appraisal of the diets of the groups surveyed." They pointed out also that many surveys are made for economic or social rather than nutritional purposes and urged cooperation between economists, statisticians and nutritionists "to ensure that a wide range of useful information is obtained from any survey, whatever its primary objective." In this context it is relevant to record that, in the 'Review of Food Consumption Surveys' compiled by FAO (18), of the 57 surveys from all over the world included because they were judged to be statistically and economically valid in concept and execution, only 17 provided any information on the quantities of food consumed by the families surveyed; the others concentrated on the expenditure of money on foods. Had nutritionists been able to cooperate in the planning, operation and interpretation of all the surveys, information of greater nutritional value might have been obtained.

The three fundamental points which have to be settled before any survey of food consumption is started are the length of time of the survey, the means of measurement of food consumption and whether the survey should be prospective or retrospective.

Surveys have been conducted over many different periods of time. Norris (46) recommended that in the Western world each survey should cover at least 7 consecutive days and that surveys should be made at each season of the year to get an adequate description of the usual food consumption, but she pointed out that a 7-day survey may not be appropriate in a country where social customs are different and that some other suitable length of time may have to be determined according to some such factors as the local pay day, or religious festivals or feasts. Where no definite cycle exists she suggested a 10-day survey to facilitate subsequent calculation. In the United Kingdom it is considered essential to include the week-end in any survey of food consumption, as the kind of food eaten on Sunday differs from week-day consumption (57).

Error in measuring family food consumption may arise if the housewife changes her purchasing habits because of the survey itself. Evidence that this can sometimes happen is supplied by the records obtained from the British National Food Survey of the energy value of foods purchased during the week of survey by elderly women who live alone. In a study of such women over the age of 55 (722 in all) who provided survey records between April 1953 and March 1954, Baines and Hollingsworth (2) found that in each decade of life from 55 onwards the energy value of the food they obtained for consumption was some 1,000 cal per head per day greater than requirements calculated according to the recommendations of the FAO Committee on Calorie Requirements (17). More recent analyses have confirmed the finding, which is not yet fully explained. Further investigations have, however, suggested that wastage is not the main explanation, and that this small group of consumers may react somewhat atypically to investigation by buying much more of certain foods during the week of the survey than they intend to eat.

One way of examining distortions of expenditure is to conduct similar surveys for different lengths of time. Presumably overspending for 1 week would not be continued for a month or more; further, errors of omission might be accentuated in the second week. For both reasons one would expect weekly records to give averages greater than those based on a longer period, though, in the absence of a criterion external to both, one could not say which is the more accurate. Prais (48) has made a study on these lines in Israel. A sample of about 3,000 households was divided at random into 3 approximately equal groups, differing only in the length of time over which they recorded their food expenditure. The first group kept records of food expenditure for a month, the other 2 for the first and second fortnights, respectively. For all food, the 2 fortnightly groups recorded expenditures whose sum was 5% greater than the expenditure of the first group during the whole month, a difference which was significant at the 1% probability level. The greatest differences were recorded for sugar and oil, foods which are comparatively non-perishable.

At one time it was thought that more accurate records of weekly food expenditure and consumption would be obtained in the British National Food Survey if the larder stocks were measured by the investigator at the beginning and the end of the week of survey, and from 1942 to the middle of 1951 such measurements were made. This procedure took much of the investigator's and of the housewife's time. At the beginning of 1950 the survey was extended from the urban working class, on whom it had formerly concentrated, to the middle and upper classes, and the measurement of larder stock became even more difficult and time-consuming. Analyses showed that on the average all classes withdrew from stock more food than they put in, probably because the survey took up time which might have been spent in shopping, because the housewife's attention was drawn to stocks which she had forgotten and then proceeded to use, and possibly because she postponed shopping until the last weighing in order to save trouble. It seemed, therefore, that the recording of changes in larder stocks caused an underestimation of normal food expenditure, but probably not of the total quantity of food consumption (though the pattern of consumption may have been distorted, e.g. by the replacement of perishable foods by canned or more durable cereal foods). In addition the weighing of larder stocks appeared to add to the reluctance of housewives in the higher income groups to participate in the survey. Accordingly, in June 1951 the survey procedure was

simplified. The recording of larder stock changes was discontinued, except for a few items of home-produced food normally stored in bulk. The immediate result was an increase in food expenditure beyond what could be attributed to changes in prices and the normal seasonal changes. Subsequent analysis showed that, on average, the total quantity of food represented by combined purchases and stock withdrawals on the former system was approximately equivalent to quantities purchased or withdrawn from bulk stores of home-produced food under the new system (42). Thus, it was concluded that a more accurate estimate of the normal family food consumption could be obtained without measuring changes in larder stocks. As already stated, later analyses on the special group of elderly women who live alone have suggested that for such women—and, it appears, only for these—the omission of measurement of stock changes may lead to a considerable overestimate of food consumption.

The next point to be considered is whether food consumption should be weighed and, if so, by whom, or whether quantities should be estimated. The third point, whether the survey should be prospective or retrospective, is related to the second and will be considered with it.

Most family surveys made in recent years appear to have been based on quantitative records of the amounts of foods bought or obtained from garden or farm. Examples are the National Nutrition Survey of Ireland of 1946 (45), the food consumption surveys made in France between 1946 and 1951 (54), the Dutch nutrition survey of 1951/1952 (12), surveys made in Israel between 1943 and 1956 (Bavly, unpublished report), Polish food consumption survey of 1957 (Szezygiel, unpublished report) and the National Food Survey of Great Britain which has been in almost continuous operation since 1941, the results of which have been published in a series of reports from 1951 onwards (33–43). The most notable exception to this generalization is the survey made in the spring of 1955 by the U. S. Department of Agriculture on household food consumption in the United States of America and published in a series of reports of which No. 6 (56) gives details of the method of survey used. In this survey information was obtained from the housewife by the 'recall-list method'; interviewers used a detailed list to help housewives to recall the quantities of foods consumed during the 7 days preceding the interview and the amounts paid for purchased items. Food consumption data were thus based on quantities remembered by the housewife over 7 days. Burk (6) has compared the technique and results of this survey with those of the National Food Survey of Great Britain for the spring of 1955, stating that "National Food Survey technicians were especially curious about our reliance on recall for U. S. Department of Agriculture household food surveys. Whereas American housewives rely more and more on one big food shopping trip each week with a few extras now and then, British housewives tend to shop every day and in several different stores. Without doubt it is much easier to recall purchases made on one organized shopping trip than in a series of small purchases." She, nevertheless, drew attention to the "unknown degree of memory bias in recalling use of food in the preceding week."

It is of interest to consider one similar finding in both surveys. In the American survey the total food consumption provided 3,200 cal per person per day or 4,390 cal per nutrition unit, taken to be a man aged 25 with an energy requirement of 3,200 cal per day (21). This would suggest an overestimate of foods used, an underestimate of requirements, a steady increase in body weight of adults, a large wastage of foods or a combination of all 4 effects. The excess of consumption over requirements of nearly 40% in terms of calories has been discussed by Goodhart (24). In the British survey for the year 1955 the energy value of total food consumption was 2,640 cal per person per day or about 15% above the recommendation of the British Medical Association (5). It is necessary to find out whether both methods of surveying, and particularly the recall method, overestimate consumption, whether requirements are underestimated, a question also asked by Trémolières and his colleagues (54) particularly for sedentary groups, or whether households really waste so much food—whenever it is measured household wastage is very low, according to Leitch and Aitken (28) rarely more than 3% of the total and in the Carnegie Survey (9) 1%.

Baines and Hollingsworth (3) have recently discussed the apparent wastage of foods implied by the results of both food balance sheet and food consumption studies by comparing the energy value of British food supplies and the estimated energy requirements of the population. In 1956 total supplies at the retail level were equivalent to about 3,100 cal per head per day, compared with requirements at the physiological level of about 2,400 cal per head per day, a gap of 700 cal per head per day or a fifth of the supply. The energy requirement of the households which took part in the National Food Survey in 1956 was 2,250 cal per head per day. They obtained food amounting to 2,620 cal, a gap of over 350 cal or about a seventh. Such large gaps between supplies and physiological requirements cannot be satisfactorily explained, but their occurrence in all well-developed countries is confirmed by comparisons between estimates of the calorie value of food supplies in FAO Food Balance Sheets (e.g. 16) and of calorie requirements according to FAO recommendations (17). It is possible to reconcile the British total supply estimates at the retail level and domestic consumption recorded by the National Food Survey. It is not, however, possible to bridge the gap between either estimate of food consumption and the physiological requirement by any observed facts. It would be interesting to know whether activity is underestimated, body weight increasing or food being wasted to previously unrecorded extents.

This inability to match intake and requirement in terms of calories is a stumbling block to the use of such

estimates of food consumption for nutritional purposes. For if calories are being wasted to this extent, what of proteins, minerals and vitamins, some of which are not present in diets in such apparent abundance as calories? This question has been asked, but not answered by Goodhart (24) and, relating to a different survey, by Wilhemy, Young and Pilcher (61). In the present state of knowledge it is impossible to answer it. Inadequate information exists on the types and amounts of food wasted. Even if we had complete information on wastage, lack of knowledge about the quantities of foods eaten by different members of the family makes it impossible to use family food consumption data as more than a general indication of the nutritional condition of the family as a whole. Such data are, however, invaluable when there is any suggestion of shortage, and, if based on sound sampling of the population, can point to groups in need of more intensive investigation. If conducted by comparable methods over time, they can too be compared with records of health, also assembled over time. Attempts are being made to use the long-term records of the National Food Survey of Great Britain in this manner.

Individual enquiries. So many studies have been made on the dietary intake of groups of individuals that it is impossible to do more than mention the most important of the techniques that have been used.

Burke (7) has described the use of a dietary history as a tool in research. Her main reason for adopting this method in studies attempting to evaluate nutritional status was the fact that "clinical signs and symptoms of deficiency, alterations in patterns of growth, physical health, endurance, efficiency, frequency of illness and the like, if related to the dietary intake of an individual, may reflect either or both of the following: the *average* dietary intake of the individual for the period under consideration, and/or the nutritional status of the individual just prior to the period considered, which in itself may reflect to a high degree the previous long-time food habits. If this is the case, it is the average dietary intake over a considerable period which is of interest for correlation with clinical and laboratory findings. This can be obtained only by the dietary history method." She was careful to point out that success in obtaining useful dietary histories depends on the training and personality of the interviewer and on the adequacy of the forms used at the interview.

Wiehl (e.g. 58, 59) had earlier relied upon the dietary history method, which according to Leitch and Aitken (28) had almost certainly produced underestimates of food intake. Later workers, for example Trulson, Hegsted and Stare (55), obtained information on the previous 24-hour intake of their subjects by interview and by means of records prepared from memory by their subjects and concluded "Since the findings of this study compare reasonably well with those of other studies done by various methods, we believe the data present material which shows trends in the food practices of the low-income groups, and that the study can serve as a base line for further research and investigation of nutritional status of the population."

Babcock, Church and Gates (1) attempted to assess the nutritional status of industrial workers by means of dietary, blood and physical measurements. The dietary intake was assessed from a record of the usual food intake obtained at a 30-minute interview and a 7-day record of food consumption kept by each subject over 7 days after the interview. Estimates obtained by the interview tended to be higher than the week's record. The authors attributed this to some overestimation in the interview of serving sizes 'because of a tendency of the men to make a good impression' and of frequency of servings, for example 'a tendency for the men to say they had certain amounts of milk and vegetables *every day*, when it is more probable that they consumed that much most days, and less on other days,' and in the 7-day records underestimation of the frequency of servings because of a decline in the subjects' enthusiasm for keeping records. The authors thought that the best estimate of intake lay between those obtained by the 2 methods. Unfortunately, the results of the physical and biochemical examinations were not, apparently, compared with the dietary findings: the latter appear to have been used as a basis for conclusions and dietary advice.

In an experimental study on 8 scientists, Morrison, Russell and Stevenson (44) compared the subjects' estimate by memory of what they had eaten at the previous 4 consecutive meals with the amounts of each cooked dish, weighed at the time of serving, and made from weighed ingredients, which the subjects had actually eaten. The average of the intake of calories estimated by the recall method was 94% of the average energy value obtained by evaluation of weighed foods. The results for individual subjects by recall ranged from 63 to 125% of the weighed values and in only 1 subject was the discrepancy within $\pm 5\%$.

The general consensus appears to be that a more accurate estimate of food intake can be obtained by a prospective account of the amount of food consumed, either weighed or measured, kept by the subject under investigation, probably with the help of the investigator, in a book provided for the purpose. Indeed, Leitch and Aitken (28) considered that this conclusion could almost be taken for granted. In a few British studies (14, 23, 60) it has been shown that weighed records of food intake of groups of individuals over a week will equate in terms of calorie intake to within about 10% (and sometimes much nearer) of observed energy expenditure. Another British study, the purpose of which is to investigate the possible relationship between diet, amongst other factors, and the incidence of the coronary diseases and other diseases of high morbidity and mortality in middle-aged men, is at present in progress on bank officers. In this, an attempt is being made to obtain reliable, individual dietary information which can be used, in the words of Marr, Heady and Morris (30) "as a yardstick . . . for the validation of simpler methods of assessing individual

diets that can be applied in large-scale epidemiological surveys." These workers have reported high correlation coefficients (of the order of +0.8) for certain components in the diets of 25 of their subjects measured in two weighed 7-day surveys made at intervals of 6 months. These results encourage the hope that, at least in one type of consumer, food habits are sufficiently stable to permit the use of a measurement of food consumption as a means of classification of individuals in a longitudinal epidemiological study. It is not without interest that, in their celebrated study (13) on the influence of prenatal diet on the health of mother and child, Ebbs, Tisdall and Scott used a 7-day record (weighed wherever possible, but otherwise measured) as a basis of classification into 'good,' 'fair' and 'poor' diets and for subsequent examination of obstetrical performance and health of infants.

Earlier, Darby (10) concluded that 7- and 3-day records in terms of household measures of food eaten at each meal in studies in Tennessee and North Carolina give "a true qualitative picture of the actual foods consumed by the population. Examinations of the seasonal patterns bear this out. The attempts at quantitative expression give reproducible average results."

The conclusion to be drawn from all the studies mentioned, and others, is that accurate estimates of food intake of individuals can be obtained by thorough investigators. It appears that weighing is more commonly used in the United Kingdom and measurement in terms of household measures in the United States. A possible reason for this has been suggested by Adelia Beeuwkes who, on observing a kitchen scale in the home of the author, asked if it was common for British housewives to weigh ingredients of recipes, the answer being that most British cookery books are written in terms of weights of ingredients and no standard household measures are in use. This leads to the further conclusion that the techniques of measurement to be adopted should be chosen to suit the methods of buying and measuring foods operative in the community to be studied. The question which is left unanswered is whether such accurate estimates of food intake do or do not represent the usual intake of the individual studied. Burke (7) considers it better to rely on dietary history, but no one appears to have undertaken the formidable task of establishing the truth of this belief.

The conclusion to be emphasized is that although even the most exact and representative record of dietary intake cannot be used to determine nutritional status it can, if converted as accurately as possible into nutritional value and if compared with clinical measurements and records of health, help in the task of accumulating knowledge on human nutritional requirements. On this theme, the words of Leitch and Aitken (28) are hard to improve ". . . the entire eating habits of the world constitute a mass experiment which can be investigated only piecemeal by small dietary and clinical surveys; these provide in turn the primary data for the study of requirements, and checks on the validity of interim standards. The diet of the people of most beautiful physique, most abounding energy, and least ill health is, at any given stage in our study of diet, the inspiration of and the check on our theories of optimum diet. But health, like diet, is affected by a host of social or economic compulsions, and as many undisciplined stupidities. The most interesting and difficult task that faces the student of nutrition or of social medicine is the disentangling of the results."

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Panel Summary—Nutritional Status in Man

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IT IS AN EXCEEDINGLY DIFFICULT TASK to make a summary of this discussion. The scope of the papers which we have heard this afternoon and the diversity of the questions that have been sent forward on these papers further emphasize the very great number of problems which exist in any system of evaluating dietary surveys. The point that has come out most clearly is that, for any evaluation of nutritional status, it is necessary for coordination of all types of survey.

We need the biochemical survey, the clinical survey, and the dietary survey, and a complete evaluation can only be obtained when all three are coordinated in a way which Dr. Schaefer has emphasized, when the biochemical studies are made as Dr. Bansi has suggested by simplification of methods; when, as Dr. Arroyave has suggested, the taking of samples for biochemical study is sufficiently simplified so that workers in the field can take those samples, can transport them back to the laboratory and can obtain satisfactory measurements of the different nutrients which are being studied.

In the case of the dietary surveys, the methods are very important, and the time of survey is a very great consideration. We are not able to do the kind of individual survey which Dr. Chappell reported in Britain where she studied the diet of one individual for more than a year by weighing the food intake. This is, perhaps, utmost perfection. If we are going to do individual studies we need careful studies on a sufficient number of persons, and this applies to all types of dietary study and to all types of nutritional study, by biochemical, clinical, and dietary methods. I think the discussion this afternoon has firmly underlined the need for coordination between all kinds of people concerned with nutrition in the field and between different disciplines in the office and the laboratory.

PANEL II

Proteins and Amino Acids in Nutrition

Chairman: Eliane Le Breton, France
Moderator: John F. Brock, South Africa

Dietary proteins in relation to man's health

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IT IS LESS THAN A DECADE since the United Nations agencies, WHO and FAO, discovered *a*) kwashiorkor as an entity; *b*) its origin in protein malnutrition; and *c*) its vast worldwide prevalence and importance (10). In human nutritional studies and in international public health this has been a protein decade. It is profitable to take stock of rapidly advancing knowledge and the possibility of its application to the welfare of man.

The stocktaking can be introduced by asking 2 mutually dependent questions:

1) How is man's health adversely affected by wrong quantity or quality of proteins and how can the wrong trends be recognized and identified? This question is inseparable from the second question since the term 'wrong quantity and quality of proteins' assumes that there is a minimum, a maximum and a desirable range of intake of a defined protein-rich food for a 'reference man,' and that equivalent figures are given for alternative protein-rich foods.

2) What quantity and quality of proteins does man require at different ages and under differing environmental circumstances in order to achieve optimum health—or in more direct words, to ensure that his health could not be improved by altering either the quality or quantity of protein in his diet?

It is unlikely that either question can be answered without answering the other or that they will be answered in our present discussion. But the discussion should show up the gaps in our knowledge and define the directions for future research. This will be the task of the summariser.

It is my task as a physician-internist to open the discussion by attempting to answer the first question in such a way as to justify the importance of seeking an answer to the second question with speed and determination.

Firstly, it is necessary to define some words and concepts. I attempted this in my Humphry Davy Rolleston Lectures to the Royal College of Physicians of London in 1959 (1) and summarised the matter again in 1960 in a Symposium on Humanity and Subsistence at Vevey, Switzerland (15).

After defining the following terms:
Nutrition and Constitution;
Malnutrition, Undernutrition and Overnutrition;
Nutrients and Foods (foodstuffs);
Recommended Allowances, Minimum and Optimum Requirements of Individual Nutrients;
Dietary and Conditioned Malnutrition (and their vicious circle);
and discussing the role of nutrition in disease of multiple aetiology, I suggested that the clinical results of malnutrition could be classified under four groups:

1) Subnutrition (subclinical malnutrition)
  a) Without demonstrable biochemical abnormality
  b) With demonstrable biochemical abnormality
2) Reversible clinical syndromes of malnutrition
3) Irreversible structural damage
4) Constitutional susceptibility (diathesis) from chronic malnutrition

This approach can conveniently be applied to the consideration of the effects of dietary protein deficiency or imbalance.

PROTEIN AND PROTEIN FOODSTUFFS

The last decade has seen a shift of emphasis away from an earlier too exclusive pre-occupation with vitamin nutrients to a more realistic appraisal of the importance of the quality, or make-up, of proteins, fats and carbohydrates. These 3 can no longer be treated as nutrients but must be regarded as constellations of nutrients. The same decade has shown the great economic importance of protein malnutrition; that is of malnutrition resulting from too great a dependance on starchy foodstuffs at the expense of protein-rich foodstuffs. Protein malnutrition[1] is most evident in the post-weaning years (kwashiorkor) but it is reasonable to suspect that it may have effects on constitutional susceptibility at all ages. Because starchy foodstuffs and protein-rich foodstuffs are variable constellations of

[1] The third Joint FAO/WHO Expert Committee on Nutrition (1953) produced the following definition of protein malnutrition: "This term cannot at present be defined within narrow limits. It is used here to indicate in general a state of ill health occurring where diets are habitually poor in protein, while they are more nearly adequate in calories. Clinically, protein malnutrition is most easily recognized when there is a relatively high intake of calories from starchy foods together with an insufficient intake of protein. The concept includes the effects of deficiency in the quantity of protein consumed, of imbalance of amino acids, and deficiency of factors, such as vitamin B_{12}, commonly found in foods in association with animal protein and concerned with protein metabolism."

nutrients (including amino acids, sugars, fatty acids, vitamins and minerals) the clinical picture of kwashiorkor varies from region to region according to the prevailing local staple foodstuffs.

PROTEIN DEFICIENCY AND PROTEIN MALNUTRITION

An infant suckling at an inadequately lactating breast suffers from deficiency of a balanced diet. Apart from this instance, such a balanced undernutrition is very rare. Not only are carbohydrates, proteins and fats balanced as between themselves but each one is balanced in itself, e.g. the amino-acid pattern (aminogram) of the human milk is perfectly balanced for the infant. Provided lactation is not too seriously impaired, nor the undernutrition complicated by bacterial infection or other environmental stress, the infant's growth will be retarded but lost ground may be made up when lactation improves.

At weaning the child can be placed on an artificial formula based on, e.g. cow's milk, and gradually weaned on to mixed feeding with healthy development. This is achieved in privileged communities which have sound medical and nutritional services and education. It is probably also achieved in primitive communities where there is no population pressure.

When population pressure develops, hunger demands a full stomach and agriculture is diverted towards foods such as cereals and tubers which have a high yield of calories per acre. The scene is now set for protein malnutrition.

Proteins are variable combinations of amino acids but protein foodstuffs always contain many other nutrients as well. If foods are expressed in terms of grams of protein yielded per hundred calories (fig. 1) they fall naturally into 2 groups which can be called, respectively, low-protein and high-protein foods. The low-protein foods include all the cereal grains, yielding about 2–3 g of protein per hundred calories, i.e. they are marginal for human protein requirements expressed in quantity. The roots and tubers all fall below 2 g/100 cal., e.g. cassava flour (manioc, arrowroot) yields under 20 g of protein per 2000 calories, and its aminogram is unbalanced. If these foods are used for the diet of weanling children without enrichment by high protein foods, calorie requirements will be met but protein intake will be quantitatively and/or qualitatively deficient. An adequate calorie intake can economise a considerable amount of protein but there is a limit to this process of economising, especially if the aminogram of the protein is not perfectly balanced. Aminogram imbalance is characteristic of all vegetable proteins (e.g. wheat is deficient in lysine, maize is deficient in lysine and tryptophane). A supplement of a small amount of protein with well balanced aminogram (e.g. milk) will extend the value of the cereal diet considerably. Failing such compensation, there will be protein malnutrition. In the post-weaning child, the result is kwashiorkor.

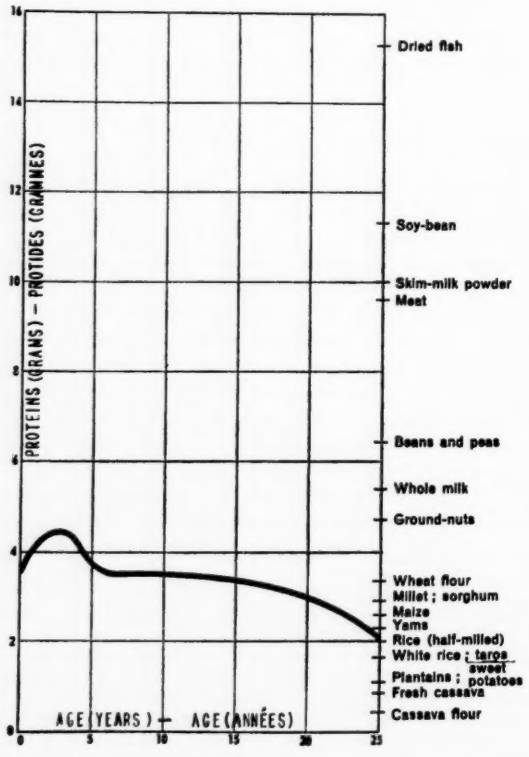

FIG. 1. Protein requirements per 100 cal., according to age. The yield of protein per 100 cal. from some common foodstuffs is indicated on the right-hand side of the figure. (From Brock, J. F. and M. Autret. *Kwashiorkor in Africa.* Monograph Ser. World Health Organization, No. 8.)

The name kwashiorkor is suitable because it means 'the deposed child' (4), i.e. the child which has been deposed from the breast by the next succeeding sib on to a diet rich in starch calories but deficient quantitatively or qualitatively in protein. The clinical picture of kwashiorkor will vary from place to place in the world according to the nutrient pattern of the prevailing starchy diet and other environmental stresses (e.g. infections and tropical parasitism). There will be deficiency of many nutrients but the most-limiting nutrient will be a group of essential amino acids. Our group in Cape Town (2, 5) has shown that initiation of cure in kwashiorkor can be achieved on a mixture of synthetic amino acids with glucose, a vitamin mixture and suitable electrolytes. When the vitamin mixture is omitted, cure is initiated, although admittedly more slowly; obviously cure can not be consolidated without vitamins. No combination of nutrients will achieve this initiation of cure unless it contains a suitable pattern of amino acids. The term syndrome pluricarencial (7) is therefore not really preferable to kwashiorkor, since it conceals the fundamental importance of amino acids.

In underprivileged communities or among ignorant and socially depressed groups in city slums, there will be, for each case of kwashiorkor, a hundred or more cases

of mild protein malnutrition (pre-kwashiorkor), with partially impaired health and increased susceptibility to infantile disease such as gastroenteritis and respiratory infection. As age advances and physical activity and the development of teeth improve, the child will ordinarily achieve a better intake of protein and, having a lesser requirement of protein per 100 calories, it will begin to pick up the retardation of growth which it experienced during the first 3 or 4 years of life. Its general health will improve, but often full vitality and normal development will be permanently retarded through minor degrees of continuing protein malnutrition.

In terms of the opening discussion on 'Clinical Results of Malnutrition' kwashiorkor will represent *group 2*, i.e. a reversible syndrome of malnutrition. In the same community when protein malnutrition is universal, the older children up to puberty and perhaps even some adolescents and adults will represent *group 1*, i.e. subnutrition (subclinical malnutrition) in which impaired health can be corrected by better feeding. The *subgroup b*) with biochemical abnormality will be represented by those who have hypoalbuminaemia (definite or marginal). Others may show no biochemical abnormality (*subgroup a*) but balance studies on a good diet may show nitrogen retention greater than is normal for their age or class.

It is not yet clear whether, or how often, this population environment will produce examples of *group 3*, i.e. irreversible malnutrition. Infection and many other stresses will help to precipitate irreversible deterioration to death. In communities without public health and hospital services there is evidence that in untreated classical kwashiorkor the mortality may be as high as 90%. In the milder cases which recover there is no present evidence of irreversible damage. In view of the personality changes of classical kwashiorkor (8) and the electroencephalographic changes (12) it is reasonable to speculate on the possibility of permanently impaired mental capacity as a sequel to protein malnutrition, since nerve tissue is more susceptible to irreversible change than most other tissues. No facts are at present available. It is reasonable to assume also that community figures for mean height and weight of populations subsisting on kwashiorkor-producing diets which are less than the equivalent mean figures for height and weight in privileged communities may reflect irreversible retardation during the growth period. So many other factors, dietary and nondietary, enter however into this end result of underprivilege that the effect of protein malnutrition can only be conjectured.

Consideration of the effects of protein malnutrition on constitutional susceptibility is likely to be more rewarding.

PROTEIN DEFICIENCY AND UNDERNUTRITION

In introducing the subject of protein malnutrition the example was given of an infant at a poorly-lactating breast who developed balanced undernutrition. Although this type of malnutrition is likely to be rare after weaning, nevertheless undernutrition is common at all ages and it will usually be associated with deficiency of all 3 types of energy food and with many other nonenergy-yielding nutrients (vitamins, trace minerals, etc.). In other words it will be unbalanced undernutrition. This differs from protein malnutrition only in that in the latter process a group of amino acids ordinarily provided by protein-rich foods constitutes the most limiting nutrients while calories are relatively, or even absolutely, sufficient through the consumption of starchy foods. It must be obvious, therefore, that in practice every conceivable gradation and combination of deficient diets will be encountered from balanced undernutrition, through unbalanced undernutrition and malnutrition including protein malnutrition. The main justification for singling out protein malnutrition for special attention is its very wide prevalence and public health importance. Again it must be emphasized that protein malnutrition is a variable type of unbalanced malnutrition (often unbalanced undernutrition) but it has 2 distinguishing points, *a*) it is due to overdependence on starchy foods at the expense of protein-rich foods; and *b*) no combination of nutrients will achieve initiation of cure unless it contains a suitable pattern of amino acids.

NONDIETARY CAUSES OF PROTEIN DEFICIENCY

Up to this point the discussion of protein deficiency has centred on diet but this does not imply that nondietary factors are not important. Climatic stresses, psychic stresses, disturbances of the gastrointestinal tract and of metabolism all help to condition malnutrition on marginal diets. Some of the disturbances of the gastrointestinal tract and of metabolism will themselves be due originally to malnutrition and will have aggravated and been aggravated in a vicious circle mechanism. This is probably especially true of infant diarrhoea. I confidently predict that when the full story of germfree mammalian life has been worked out it will be found that malnutrition upsets the normal balance of intestinal bacterial flora, and that this in turn opens the way for invasion and multiplication of intestinal pathogens. Only in this way can the close association be explained between malnutrition and infantile gastroenteritis and diarrhoea.

CLINICAL RESULTS OF PROTEIN MALNUTRITION AND UNDERNUTRITION

In the recently weaned infant, kwashiorkor is the clinical expression of protein malnutrition, while marasmus is the clinical expression of undernutrition; in nature almost invariably unbalanced undernutrition. When the undernutrition has that sort of unbalance represented by protein malnutrition, i.e. excess in starchy foods and deficiency in protein-rich foods while at the same time calories are also markedly deficient, the clinical result has been aptly called marasmic kwashiorkor. An adequate metabolic characterisation of the differences between kwashiorkor and marasmus has long

been awaited and it is my hope that it may be provided at this Congress. Marasmic kwashiorkor will presumably show metabolic and biochemical disturbances in respect of nitrogen intermediate between those of marasmus and those already defined for kwashiorkor.

One metabolic difference which is inherent in the definitions given but which has not yet been put to adequate experimental test is the response to nonprotein calories. It was stated earlier that in classical kwashiorkor no combination of nutrients would initiate cure unless it contained a suitable pattern of amino acids (2, 5). In undernutrition the most-limiting dietary factor is calories and the infant will presumably take calories from any calorie-yielding food, presumably from carbohydrate, fat or protein indifferently. The result will be growth and improvement, which must however soon be slowed and halted by the absence of the most-limiting nutrient. In marasmic kwashiorkor some growth and improvement may be expected on provision of, say, calories in the form of starchy foods, but the growth and improvement may be expected soon to be slowed and halted if the most-limiting amino acids are not provided.

CLINICAL RECOGNITION OF CHRONIC PROTEIN DEFICIENCY

The clinical picture of kwashiorkor has been defined (4, 17). From what has been said about both starchy and protein-rich foods as constellations of nutrients it is inevitable that the clinical picture should vary from region to region according to the type of staple food consumed. Nevertheless, there is a basic clinical pattern which is unmistakable. It can be briefly summarised as follows: at a variable number of months after weaning, growth and development are slowed; variable and nonspecific disturbances of colour and texture occur in skin, hair and mucous membranes; eventually progressive deterioration in all functions is precipitated by gastrointestinal disturbances (especially diarrhoea) and other vicious circle complications; the severe picture is dominated by oedema and irritable mental apathy. Full investigation and autopsy at this stage, which is irreversible without a diet containing suitable protein or amino acids, discloses disturbances of every tissue; most characteristic are fatty liver, intestinal and pancreatic atrophy, decline in many enzyme systems, hypoalbuminaemia and deficit in nitrogen and potassium metabolism. Infective, parasitic and other complications may be present, but analysis of the dietary and medical history indicates that the major cause has been the use of a starchy diet without suitable protein-rich supplements.

Evidence of vitamin deficiency is often present but is secondary. The classical clinical picture merges, when starch calories have also been deficient, through the variant described as marasmic kwashiorkor towards the picture of marasmus. An equivalent clinical picture may occasionally be encountered at all ages, but after the age of 5 it becomes uncommon and more difficult to differentiate from undernutrition and famine oedema.

METABOLIC AND BIOCHEMICAL RECOGNITION OF PROTEIN DEFICIENCY

From this clinical description and from the concepts of protein malnutrition and unbalanced undernutrition it is apparent that metabolic and biochemical tests are needed to identify the effects of deficiency of nitrogen or of specific amino acids among the mixed picture of malnutrition which results from deficiency of protein-rich foods with its associated deficiency of vitamins, minerals and even calories.

The clinical pictures of deficiency of most of the vitamins have been known for many decades, e.g. scurvy, pellagra, rickets, etc. A few have only recently been defined, e.g. pyridoxine deficiency convulsions. In the case of other vitamins, the precise clinical manifestations of deficiency are still uncertain. Laboratory studies of the level of vitamins in urine and blood have given greater precision to clinical diagnosis and with many vitamin deficiencies it is possible at least to exclude (if not to confirm) by laboratory methods a deficiency state which has been clinically suspected. Positive identification is less easy because the precise levels which differentiate a state of low reserve from a state of definite deficiency have still to be laid down. Further laboratory elaboration is possible through the study of abnormal metabolites, e.g. pyruvic acid and *n*-methyl nicotinamide with or without a loading stress.

In contrast to this body of knowledge our recognition by clinical or laboratory means of states of nitrogen and amino-acid deficiency is still very incomplete.

The metabolic and biochemical abnormalities of kwashiorkor obviously give the clue. Some of these, however, appear to be nonspecific results of mixed malnutrition, e.g. low levels of cholinesterase in the serum. Others are the result of deficiency of vitamins ordinarily provided by protein-rich foods but not directly the result of amino-acid or nitrogen deficiency. The latter results are the ones in which we are more interested.

Plasma amino acid levels. Perhaps the most logical approach to this subject is in the recent work of Longenecker and his colleagues (11) who have concentrated on defining the most-limiting amino acid in a given diet. The method can be applied to man, given sufficient resources. It is based on the postulate that when a given protein is fed its most-limiting amino acid is indicated by a fall in the plasma level of that amino acid only. The application of the method has certain limits because the body has some capacity to substitute one amino acid for another. Even the term essential amino acid has some relativity.

Urinary amino acid excretion. Abnormal quantities and patterns of amino acids in the urine are encountered in a number of malnutritional states. Efforts to establish a pattern which is diagnostic of a given form of dietary protein deficiency have, however, not yet been successful (14).

Serum and urinary urea. Low figures under both headings may be taken as evidence of low intakes of dietary

protein, at least in the absence of liver and kidney disease. They do not, however, establish a state of protein depletion.

Pattern of urinary nitrogen constituents. Platt has suggested (13) that the determination of urea and ammonia as a percentage of total urinary nitrogen in a single morning specimen provides all the information necessary to assess protein nutritional status. Our own urinary nitrogen partition studies (H. E. Schendel *et al.* In preparation, 1960.) also indicate that the excretion of urea is reduced in this state. We were unable, however, to confirm their observation that the absolute excretion of ammonia is increased. Its excretion appears to be roughly constant throughout treatment and decreases as a percentage of total urinary nitrogen only because the absolute urea excretion increases.

Nitrogen balance. The dynamic study of nitrogen balance can be most rewarding. Hansen (9) and Truswell (16) have recently completed from our Medical School exhaustive studies of its application, limitation and interpretation, respectively, for infants and for adults. There is no doubt that such balances can identify the presence and severity of nitrogen deficiency and can be used to measure the nutritive value of the proteins of foods and the effect thereon of protein and amino-acid supplements.

Marginal hypoalbuminaemia. For ordinary practical purposes the simplest biochemical test for protein deficiency is to measure the level of serum albumin. In the absence of losses from the kidney, skin, or intestinal mucosa (exudative enteropathy) or of impaired liver function, a fall in serum albumin is presumptive evidence of dietary protein deficiency. In metabolic work on convalescent kwashiorkor cases we have found it a most reliable index of improvement or deterioration. Unfortunately, significant depression is a relatively late sign of protein depletion. We have therefore examined the significance of 'marginal hypoalbuminaemia.' Brock (3) compared the relationship of marginal hypoalbuminaemia to protein deficiency with the relationship of marginal haemoglobin levels to iron deficiency. When replacement iron therapy (e.g. 9 grains of ferrous sulphate per day) leads to a rise in individual or group mean haemoglobin levels from the zone 10–12 g % to the zone 12–14 g % most workers will accept this as evidence of iron deficiency. It is then logical to conclude, in the absence of evidence of disturbed iron metabolism or of abnormal haemoglobin losses, that the dietary iron intake of a group of people with haemoglobins in the zone 10–12 g % was suboptimal. In another communication (H. E. Schendel, in preparation, 1960), an attempt is made to define marginal serum albumins by one laboratory method. In 71 children with acute kwashiorkor, the mean serum albumin was 1.80 g % ±1 S.D. = 1.31–2.29. In 51 children whose serum albumin had settled to a plateau after satisfactory treatment (ceiling level) the mean figure was 3.84 ± 1 S.D. = 3.50–4.18. In a group of 41 slightly older children who were followed on a protein-deficient diet until 3 of them showed clinical evidence of protein depletion, the mean figure was 3.15 for the remaining 38 was 3.15 ± 1 S.D. = 2.78–3.52. This latter range is very significant when compared with the highest figure for the acute kwashiorkor series and the lowest figure for the cured cases (ceiling level), respectively, 2.29 and 3.50. This is a remarkable correspondence and suggests that by the methods used marginal hypoalbuminaemia for young children is of the order 2.8–3.5. If the analogy with marginal haemoglobin figures and iron deficiency and repletion holds, then it can be concluded that children within the range 2.8–3.5 by our method who are not suffering from abnormal protein losses and who have not liver disease to interfere with synthesis, will improve their serum albumin level by appropriate protein feeding.

Extracellular albumin pool. A promising approach is the measurement of dilution of injected tagged albumin in the intravascular and extravascular-extracellular pools of albumin (6). Recent application of this method to our own clinical material leads us to believe that this may be a sensitive index of early protein depletion. Our material is, however, still incomplete.

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The ideal aminogram

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A STUDY of the nutrition and metabolism of dietary proteins involves a number of variables such as the caloric intake, the nitrogen intake, the pattern of amino acids provided by the diet, vitamin and mineral intakes, and the physiological state of the individual. The following discussion, however, will assume that the limiting variables to the utilization of dietary protein are the nitrogen intake and the pattern of amino acids presented to the body by the diet. The possibility of developing a reference pattern for evaluating dietary proteins will be considered, a reference pattern that may be called, or made a part of, an aminogram.

NITROGEN INTAKE

Nitrogen intake is listed as the first important variable because the synthesis and maintenance of tissue proteins can be expressed most simply as a nitrogen balance. Positive balance reflects growth of new tissue protein or repletion of old, zero balance or equilibrium can be said to represent an attempt to maintain the status quo and negative balance reflects loss in body nitrogen. These concepts are illustrated in figure 1 for growth and maintenance of body nitrogen in boys. Positive balance at different ages was calculated by assuming a certain rate of growth at each age with a constant body composition. The amount of nitrogen required for maintenance was calculated by assuming the loss of body nitrogen to be equivalent to 2 mg per basal calorie, an equivalence suggested by Terroine (74) Sorg-Matter (73) and others. A similar value for maintenance was used for man by Bricker, Mitchell and Kinsman (16) and by Hegsted (31, 32) to estimate basal requirements for nitrogen in children and adults. The values plotted in figure 1 were taken in part from a report of the Food and Nutrition Board (26). The data were presented to emphasize the importance of the growth in maintenance as well as in new tissue proteins. The area representing maintenance will, of course, become relatively constant in an adult so that the lower line in negative balance in figure 1 will become parallel to the X axis. This line could, for example, become parallel at 4.5 gm of nitrogen per day which would mean that the adult would need approximately 28 gm of ideal dietary protein per day for maintenance. The area of maintenance in figure 1, however, represents a minimum requirement, and should not be used to calculate the needs for a population of individuals. The amount of nitrogen required for maintenance increases with the magnitude of the protein reserves and labile nitrogen pool, so that some individuals would need 5 gm of nitrogen per day or 32 gm of an ideal protein for maintenance. Allowing for 10% wastage in the gastrointestinal tract, the needs would be increased to 35 gm of protein. Assuming a biological value of the dietary protein to be 70 instead of 100, the daily intake for maintenance would be increased to 50 gm. Other individuals may require more than this amount for maintenance, others may need less, but the optimum is unknown. Thus the determination of dietary protein requirements is dependent upon knowledge of the magnitude and function of the protein reserves and labile nitrogen as well as of the amino acid pattern presented to the body.

PROTEIN RESERVES

Emphasis is placed upon the concept of protein reserves because of the dynamic interrelationship between many of the cellular proteins and the so-called non-protein labile nitrogen which includes the metabolic pool of amino acids (3). Cellular proteins are not stored in special compartments to be utilized as reserves in the same sense as fat is stored. Cellular proteins, however, do contribute amino acids to a metabolic pool and if the diet is deficient in nitrogen, the body is depleted in many tissue proteins. Those proteins that can be depleted to contribute amino acids to the metabolic pool have been considered as reserves. Plasma albumin, for example, is a reserve that is depleted during a period of protein starvation but some of the plasma globulins are not reduced, even tending to increase in the depleted individual. This does not mean, however, that the globulins are static. They may be in a dynamic state being hydrolyzed and resynthesized but the amino acid pool may be utilized to maintain these globulins more than the albumins. Many cellular proteins are parts of enzymes systems, so that depletion in so-called protein reserves results in a reduction in activity of some enzymes system but others may increase (51, 76–78, 82). Data have been presented to demonstrate that the proteins of the brain are resistant to depletion, those of the heart less so, the kidney still less, while a number of cellular proteins in the liver are easily depleted (78). The possibility exists that some cellular proteins may function as reserve

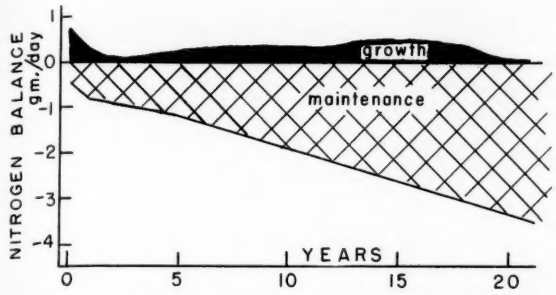

FIG. 1. An estimate of nitrogen retention for growth and maintenance in boys.

supplies for amino acids during periods of depletion either from illness or starvation. Attention must be centered, therefore, on the amino acid metabolic pool and cellular protein synthesis.

CELLULAR PROTEIN SYNTHESIS

A metabolic amino acid pool is an essential component of modern concepts of cellular protein synthesis. A soluble ribonucleic acid (S-RNA) is thought to pick up activated amino acid molecules to put them in proper sequence upon nucleic acid templates in particulate matter such as the microsomes (19, 34). Each type of cellular protein is characterized by the arrangement of amino acids along the peptide chain which emerges or rolls off the templates to be coiled and bound into specific structures. It has been suggested, for example, that there is a specific microsomal ribonucleic acid protein for each type of cellular protein synthesized. It is also possible that one microsomal machine could shift its gears, as it were, to produce a number of patterns to polypeptide chains, shifts that could be correlated with internal environmental changes. The principal point to be made here, however, is that an amino acid pool may be a part of the concept of synthesis, a pool that must come from amino acids of dietary origin and from the hydrolysis of cellular proteins. Evidence is accumulating to demonstrate that free amino acids in the plasma can be used to reflect the utilization of these acids for protein anabolism throughout the body (49).

A possible correlation may exist between ribonuclease activity and the utilization of RNA in protein anabolism. Data have been obtained, for example, to demonstrate an increased ribonuclease activity with reduced protein intake and thereby protein anabolism in the rat (82). Serum ribonuclease activity, relative to the plasma proteins, increased from 5.0 ± 0.25 to 7.3 ± 0.21 to 18.2 ± 0.60 units as the casein content of the diet fed to rats was reduced from 30 to 12 to 0%. Similarly liver ribonuclease activity increased from 0.51 ± 0.03 in animals fed the 30% casein diet to 0.61 ± 0.03 in those fed the diet containing 12% casein to 0.99 ± 0.08 units in rats fed the protein-free diet. On the other hand, RNA phosphorus decreased from 27 ± 1.4 μg/gm of liver in animals fed the diet containing 30% casein to 20.2 ± 0.6 in animals fed 12% casein to 13.9 ± 0.7 in those fed the protein-free diet. Rats were also fed a diet containing 40% wheat gluten to obtain approximately the same growth as those fed 12% casein. Rats fed the high wheat intake developed a relatively high serum ribonuclease activity (11.3 ± 0.5), liver ribonuclease activity (0.84 ± 0.4) and low liver RNA phosphorus (19.1 ± 1.1 μg/gm of liver). Thus in the rat there may be a homeostatic mechanism for protein anabolism which is correlated with the ribonuclease activity and the RNA structures of protoplasm.

Emphasis has been placed upon a metabolic pool of free amino acids which contribute to protein anabolism and catabolism in a dynamic system of interchange. Plasma proteins and polypeptides, fed intravenously, however, can be utilized by the body (2, 79, 81). Whipple and associates have emphasized the role that plasma proteins may play in supplying the building material for synthesis of cellular proteins (79). Possibly plasma proteins may be converted to tissue proteins without complete breakdown into amino acids (81) although there is evidence for complete hydrolyns of albumin before utilization (18). There is also the possibility that plasma proteins represent a unique pathway to bring amino acids to specific tissue metabolic pools of amino acids. Evidence has been presented, for example, to indicate preferential utilization of plasma proteins over free amino acids by tumor tissue (17, 33, 60).

THE ESSENTIAL AMINO ACID PATTERN

The high nutritive value of egg albumin for growth is illustrated by the data recorded in table 1 (9). The grams of amino acid available and the total nitrogen required for 50 grams gain in body weight are summarized in this table. The amino acid intakes which are underlined are taken as estimates of the minimum values within experimental error. The minimum values in the last column represent averages of the underlined. Adding methionine to casein increased the nutritive value of casein for growth to be equivalent to egg albumin with the intake pattern of all of the essential amino acids in casein approaching the pattern for egg. Wheat gluten is primarily deficient in lysine. Adding lysine to wheat gluten increased the nutritive value so that supplemented gluten almost equaled the value for casein. All but lysine, the total sulfur amino acids and valine were in excess in the supplemented wheat gluten. These results emphasize the importance of correcting the most limiting amino acid first in any supplementing procedure. Adding lysine, for example, was sufficient to bring the intake of the other deficient amino acids close to the value supplied by the intakes of egg. Compared to the egg, cottonseed meal is primarily deficient in isoleucine, leucine, lysine and total sulfur amino acids. Adding lysine or methionine to cottonseed meal did not improve the nutritive value. Adding both, on the other hand, raised the nutritive value of supplemented cottonseed meal near to that of casein. All but isoleucine, leucine,

TABLE 1. *Grams of Total Nitrogen and Amino Acid Available from Various Dietary Protein Sources Which Result in 50 Grams Gain in body Weight per Rat (9)*

	Egg	Casein + Methionine	Casein	Fish	Cotton Seed + Meth + Lys.	Soybean	Wheat Gluten + Lys.	Cotton-Seed + Lys.	Cotton-Seed	Wheat Gluten	Min. Values
Total nitrogen	1.7	1.7	2.5	2.5	2.8	3.0	3.0	4.0	4.0	8.0	1.7
Arginine	0.63	0.41	0.61	1.07	1.95	1.35	0.69	2.80	2.80	1.8	0.41
Histidine	0.25	0.32	0.47	0.26	0.46	0.74	0.37	0.66	0.66	1.0	0.25
Isoleucine	0.68	0.67	0.98	0.79	0.68	0.98	0.85	0.95	0.95	2.3	0.68
Leucine	0.93	1.16	1.56	1.18	1.04	1.45	1.35	1.48	1.48	3.6	0.93
Lysine	0.76	0.86	1.30	1.37	1.00	1.18	0.80	1.48	1.07	1.0	0.80
Phenylalanine	0.63	0.57	0.84	0.57	0.92	0.92	0.95	1.30	1.30	2.5	0.59
Tyrosine	0.35	0.55	0.80	0.40	0.48	0.60	0.52	0.68	0.68	1.4	0.37
Total aromatic	0.98	1.12	1.64	0.97	1.40	1.52	1.47	1.98	1.98	3.9	0.96
Cystine	0.31	0.04	0.06	0.21	0.28	0.33	0.44	0.41	0.41	1.1	
Methionine	0.44	0.62	0.65	0.45	0.64	0.26	0.31	0.34	0.34	0.8	
Total sulfur	0.75	0.66	0.71	0.66	0.90	0.59	0.75	0.75	0.75	1.9	0.65
Threonine	0.52	0.48	0.70	0.70	0.62	0.77	0.52	0.88	0.88	1.4	0.51
Tryptophan	0.12	0.10	0.15	0.15	0.20	0.26	0.14	0.29	0.29	0.4	0.11
Valine	0.80	0.78	1.15	0.81	0.86	0.98	0.80	1.33	1.33	2.1	0.81

and valine were in excess in the supplemented cottonseed meal. All of the amino acids were in excess when unsupplemented cottonseed meal or wheat gluten were fed in sufficient quantities to produce 50 grams in weight in the rats. Such excesses of limiting essential amino acids may be the result of inhibitions to transportation and distribution associated with larger excesses of other essential amino acids. Excess essential amino acids, not needed for protein synthesis, must be utilized for other purposes, being converted into nonessential amino acids, and other metabolites entering pathways leading to the energy supply of the body.

Methods of measuring nutritive values are thought to estimate how closely the essential amino acid pattern of the dietary protein conforms to the pattern utilized most efficiently for protein synthesis. Block and Mitchell (13, 52), for example, compared the percentage of each essential amino acid in a dietary protein to that in egg protein. The relative amount of the limiting amino acid showing the largest deficit when compared to egg was called the 'protein score.' This score was highly correlated (r=0.83) with 'Biological Values.' Oser (56, 57), however, pointed out that the one most limiting amino acid may not entirely determine the 'Biological Value' but that all deficits may contribute. He proposed, therefore, an 'Essential Amino Acid Index' (EAA) which takes into consideration the ratio of each essential amino acid in a dietary protein relative to the amount in whole egg protein. Oser has written an excellent review of this method entitled "An integrated essential amino acid Index for predicting the biological value of proteins" (57). Mitchell (54) modified the 'protein score' to take into consideration these ratios by calculating a geometric mean of the deficits of all the essential amino acids relative to those in egg protein. He dropped arginine as essential and included tyrosine with phenylalanine and cystine with methionine. Oser pointed out the limitations of this method of estimating nutritive values from amino acid analyses, chief of these being the rate of digestion and absorption of the amino acids from the intestine into the blood stream. Sheffner *et al.* (58) attempted to correct for differences in digestion by calculating the EAA for the pattern of essential amino acids in enzymatic digests of proteins. They called this the 'Protein Digestion Residue Index' (PDR).

The suggestion is often made that the essential amino acid pattern in human milk would be the best reference pattern for growth in humans (27). Such a standard was recommended by Kuhnau (40) and Nehring and Schwerdtfeger (55). There is the possibility, however, that the reference pattern should be altered to meet different physiological needs such as growth, maintenance, repletion, wound healing, etc. Natural dietary protein sources with high nutritive values such as eggs may have excess essential amino acids, at least for some functions, thereby setting too high a standard for world food supplies. One approach to the establishment of a reference pattern would be to test the nutritive value of various patterns of amino acids fed as free amino acids. Rapid absorption of the free amino acids from the intestine could be expected, thereby eliminating the problem of variations in rate of liberation of essential amino acids from various dietary protein sources in the gut. New problems, however, are introduced. The essential amino acid mixtures often contain D as well as L forms and are not balanced in relation to the nonessential amino acids. Feeding free amino acids required an increase in caloric intake to obtain maximum retention of nitrogen (63). Nevertheless, this experimental approach to the establishment of a reference pattern is one logical way to unlock the mysteries of the utilization of different patterns of amino acids. For these reasons, among others, the Food and Agriculture Organization (FAO) Committee on Proteins (26) decided to develop a reference pattern using data from experiments where amino acid mixtures had been used to determine the requirements for maintenance and growth in humans (1, 20, 35, 36, 44–48, 50, 61–63, 68–72).

The FAO pattern of amino acids is illustrated in table 2 by presenting each amino acid as a ratio to threonine. Similar ratios have been calculated for the proteins of human milk and for egg and these are recorded with the FAO pattern under reference patterns. The ratios of amino acids derived from human milk and from egg are very similar to the FAO except for tryptophan which is higher in the FAO pattern than in the other two.

The pattern for growth of the rat recorded under (a) in table 2 was calculated from the data presented in table 1. The pattern recorded under (b) was calculated from data presented by Rama *et al.* (58). These authors determined the minimum requirements for growth in the rat using a casein-amino acid diet at a level of 10% protein. The ratios calculated from their data are similar to those derived from egg proteins although the values for valine, the sulfur amino acids, and total aromatic amino acids are lower in the growth than in the egg pattern. The pattern under (c) was calculated from the data of Williams *et al.* (80) who analyzed the carcass of the rat. These patterns are in remarkable agreement and correspond also for the pattern for growth in the chicken. The pattern for the chicken was obtained from the data of Fisher and associates (23, 24, 39, 42, 43) who determined it by feeding free amino acids. A survey of the literature (3) emphasizes the importance of an intake of the essential amino acids in approximately the proportions and amounts found in milk and eggs for optimum growth of tissue proteins in most animals (1). This same pattern promotes optimum repletion of depleted adult tissues. For this reason the pattern which is optimum for growth can be recommended for both young and old, even though the pattern for maintenance may differ from the needs for optimum growth.

The pattern for maintenance in man, recorded in table 2, was derived from experiments involving the feeding of different dietary proteins (22, 30). This technique for estimating the amino acid requirements in man was used also by Harte and Travers (29). Their pattern expressed with respect to threonine was histidine, 0.5; isoleucine, 1.2; leucine, 1.7; lysine, 0.8; phenylalanine, 1.4; methionine, 0.5; threonine, 1.0; tryptophan, 0.27; valine, 1.1. These patterns for maintenance in man suggest that the total sulphur amino acid and the lysine requirements may be somewhat lower for maintenance than for growth. Certainly the lysine requirement for maintenance in the rat is lower than the needs for growth, a fact first emphasized by Mitchell *et al.* (53). The marked differences that may arise between requirements for growth and maintenance can be illustrated by the data for the chicken reported by Fisher, Leveille and associates (23, 24, 39, 42, 43). The pattern for maintenance in the chicken is arginine, 1.6; isoleucine, 1.0; leucine, 1.7; lysine, 0.4; total aromatic, 0.8; total sulfur, 1.2; threonine, 1.0; tryptophan, 0.2; and valine, 0.8. On the other hand, the pattern for maintenance in the dog, illustrated in table 3 and derived from published data (3–6), are thought to be essentially the same as the pattern for growth.

These patterns in table 2 can be converted to intakes through multiplication by appropriate factors. To calculate minimum intake of an ideal dietary protein for maintenance in man expressed as mg amino acid per day per kg of body weight, multiply the values in the maintenance pattern by 19.5. Similarly, for maintenance in the dog, multiply the values by 44. To calculate grams of amino acids needed for the growth of 50 grams in body weight in the rat, multiply the growth pattern for this animal by 0.44.

Recently several experiments have been published to test the value of the FAO reference pattern as a method of estimating amino acid deficiencies in diets of animals as well as the diets of man. Howe *et al.* (38), for example, studied the growth-promoting properties of essential amino acid mixtures patterned after the FAO reference standard and mixtures of essential amino acids patterned after casein and cottonseed flour. Their results demonstrated that the essential amino acid mixture, patterned

TABLE 2. *Essential Amino Acid Patterns for Growth and Maintenance* (9)

	Reference Patterns			Growth				Maintenance		
				Rat						
	Human milk	Egg	FAO	*a*	*b* (58)	*c* (80)	Chicken (23)	Man	Dog	Rat (12)
Arginine	0.9	1.2		0.8		0.5	2.0			
Histidine	0.5	0.5		0.5	0.4	0.6	0.5		0.5	0.4
Isoleucine	1.2	1.3	1.5	1.3	1.1	0.9	1.0	1.4	1.7	2.5
Leucine	2.0	1.8	1.7	1.8	1.4	1.9	2.3	1.7	2.6	1.2
Lysine	1.4	1.5	1.5	1.6	1.7	1.7	1.8	1.1	1.6	0.7
Phenylalanine	1.0	1.2	1.0	1.2		0.95	0.8	1.1	1.1	0.6
Tyrosine	1.1	0.6	1.0	0.7		0.75	0.8	0.7	0.9	
Total aromatic	2.1	1.8	2.0	1.9	1.4	1.70	1.6	1.8	2.0	
Cystine	0.4	0.6	0.7			0.39				
Methionine	0.5	0.9	0.8			0.44				1.0
Total sulfur	0.9	1.5	1.5	1.3	1.0	0.83	1.4	0.7	1.3	
Threonine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Tryptophan	0.36	0.24	0.5	0.22	0.22	0.20	0.25	0.25	0.29	0.35
Valine	1.4	1.5	1.5	1.6	1.1	1.4	1.3	1.5	1.5	1.4

after the FAO reference, promoted somewhat greater growth than a similar mixture with the essential amino acids patterned after casein, provided the percentage of essential amino acids in the two mixtures was the same. The protein efficiencies of the FAO pattern and the casein pattern were equal at a lower essential amino acid content but the FAO pattern still supported more rapid growth when the diets were fed ad libitum. The percentage of essential amino acids needed for growth and maintenance in man has not been finally determined but this percentage may be somewhat less than those found in some of the food proteins (36). The protein efficiency of the FAO pattern was not altered by decreasing the tryptophan content by 33 to 50% or by increasing the lysine by 22%. Thus the FAO pattern did support excellent growth in the rat provided the percentage of essential amino acids was adequate. This importance of maintaining an adequate intake of the essential amino acids relative to the non-essentials was pointed out by Harper and Kumta (28).

The essentiality of the non-essential amino acids should be considered briefly. This concept states simply that certain non-specific dietary sources of nitrogen, particularly the non-essential amino acids, are more effective in meeting the non-specific nitrogen requirements than are the essential amino acids (41, 59). Thus, if the essential amino acids are fed at levels which meet their requirements for protein anabolism and other specific purposes, additional nitrogen retention will be obtained if more of the essentials are added to cover the needs for non-essential amino acids. However, a greater amount of retention will result if additional nitrogen is given in the form of non-essential amino acids such as glutamic acid, alanine, etc. A single non-essential amino acid may not meet the needs for all of them as well as a mixture. Excess glycine, for example, gives an abnormal response in the rat. There may be interrelationships between essential and non-essential amino acids such as the one existing between glycine, arginine and methionine. Glycine and arginine form guanidinoacetic acid which is methylated through methionine to form creatine. Indeed, the toxicity of excess methionine can be overcome by adding glycine to the diet in the presence of adequate arginine (5). Mention should also be made to the antagonism which may develop between abnormal mixtures of amino acids of similar structures, antagonisms that may be associated with orientation with respect to surface or transport mechanisms (21). These and other abnormalities resulting from shifts in amino acid patterns may be characterized as imbalances (28).

In another experiment, Howe *et al.* (38) fed the rats a limited intake of casein supplemented to the FAO reference. This casein-amino acid mixture was nutritionally equivalent to the FAO reference amino acid mixture. These results can be interpreted to mean that casein is digested in such a way that utilization of the amino acids is the same whether they are fed free or combined in part as casein. Other experiments demonstrate that supplementation of easily digested proteins with free amino acids gives a good response nutritionally (25, 37, 64). Indeed, the assumption is usually made that the pattern of amino acids presented to the body through digestion can be estimated from the chemical analysis for the amino acids in the dietary protein. However, such a close correlation between the pattern determined by chemical analysis and the pattern presented to the body through digestive and absorptive processes need not always hold. This correlation has been improved and also made worse at times by technological processes (3). Possibly mixtures of dietary proteins will be found that have higher nutritive values than those calculated from the potential pattern recorded by chemical analysis, a response that could result from variations in rates of liberation of amino acids by digestive processes. Lower nutritive values, than those calculated, could also be found experimentally. For example, Howe *et al.* (38) fed to rats a limited intake of cottonseed flour supplemented with free amino acids equivalent to the FAO reference amino acid mixture. The protein efficiency of the supplemented cottonseed flour was inferior to the unsupplemented flour. These results could be interpreted to mean that the free amino acids were absorbed so much more rapidly than those liberated through digestive processes that an imbalance developed.

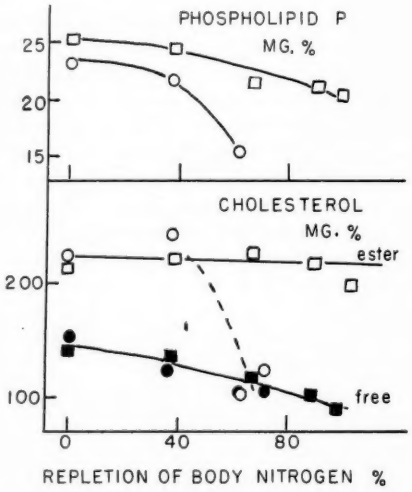

FIG. 2. Effect of repletion of depleted body nitrogen in the adult dog on a hypercholesterolemia and hyperphospholipedemia. Circles represent repletion with casein; squares represent repletion with wheat gluten.

The studies of Scrimshaw, Behar, Bressani, Wilson, Viteri, Arroyave and others (14, 15, 65, 66) at the Institute of Nutrition of Central America and Panama (INCAP), are good examples of the use of the FAO reference pattern for the experimental approach to supplementation of the diets of children. These studies have demonstrated that the nutritive value of corn protein, as measured by nitrogen balance in children, can be improved by supplementation with lysine and tryptophan and by the further addition of isoleucine to improve the isoleucine/leucine ratio (21). Similar results

were obtained by Truswell and Brock (75) in their studies on the effect of amino acid supplementation on the nutritional value of corn protein fed to human adults. Recently, Bressani *et al.*(15) have reported on the effect of amino acid supplementation of wheat flour as measured by nitrogen balance in children. As in their previous experiments, they tested the effects of adding the limiting amino acids according to the FAO reference pattern. They found that supplementation of wheat protein to meet the FAO reference standard resulted in a sustained nitrogen balance similar to the response to milk. The major response was obtained by adding lysine, the most limiting essential amino acid in the wheat proteins. They concluded also that the methionine content of the FAO reference was too high for the wheat proteins since addition of this amino acid did not improve nitrogen retention.

Emphasis has been placed upon the importance of the proportions and quantity of the essential amino acids in the diet. It is possible, however, to overcome deficiencies of amino acids by increasing the nitrogen intake provided the deficiency is not too great. In general, the data in the literature (9, 22) suggest that if the 'Biological Value' of the dietary protein mixture is less than 60, the nutritive value of the mixture should be improved to meet the needs of a population rather than to recommend an increased intake of a low quality protein. A rather dramatic illustration of the effect of nutritive value of the dietary protein upon repletion of depleted adult tissue in the dog is illustrated in figure 2. Data were presented previously (8) to demonstrate a marked rise in plasma cholesterol, particularly the cholesterol esters in dogs which had been fed a protein-free diet until they had lost approximately 25% of their body nitrogen. Accompanying this rise in plasma cholesterol was an increase in phospholipid and in the lipid migrating electrophoretically with the plasma globulins. The lipemia in the dogs with depleted reserves was most marked when the diet was relatively high in fat and when the reserves had been severely depleted. The suggestion was made that the body metabolism was being overloaded in the presence of reduced oxidative enzymes in the liver. Repletion of the body nitrogen with casein returned the lipids to normal values. The cholesterol ester, for example, decreased rapidly after 40% of the body nitrogen which had been lost was regained. These effects of repletion upon cholesterol and upon phospholipid phosphorus are illustrated in figure 2. Repletion with wheat gluten, however, did not return these lipids to normal values even though the body nitrogen had been replaced by feeding this protein. The importance of proper balance, therefore, between essential amino acids provided by the diet is a major consideration in evaluation of any diet, but is particularly important when that diet must promote growth, repletion or repair damaged tissue.

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Biochemistry of human protein metabolism

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MUCH OF OUR KNOWLEDGE in the field of protein metabolism has been gained by experimentation with animals and even microorganisms. Its application to the human organism is not always a safe procedure. Studies on protein metabolism in man have, for obvious reasons, been restricted to observations on blood, digestive juices and excreta. This has set a limitation to our understanding of human protein metabolism. In spite of this, however, a large amount of information is available in literature on different aspects of the subject which it will not be possible to survey in the limited time and space available for this discussion. Only a few aspects which are considered to be of nutritional importance are dealt with in the following pages with a view to focusing attention on them and in the expectation that the treatment will provoke thought and action which might improve our understanding of this important subject.

FOLIN'S THEORY OF PROTEIN METABOLISM

The classical studies of Folin on nitrogen metabolism in man had a significant influence on all subsequent work on this subject. Folin (16) was the first to undertake an almost complete analysis of urine in human subjects maintained for 7 days on a constant diet. Apart from obtaining valuable information on the nitrogenous constituents of urine under controlled dietary conditions, he can be said to have laid the foundation of the technique for future studies on protein metabolism in human subjects. Folin found that on an average intake of 16.0 g N, urea formed 87.5% of the total urinary N, the rest being made up of ammonia, creatinine, uric acid and undetermined nitrogen. He further showed that the amount of urea in urine varied according to the level of dietary protein and hence the level of protein metabolism. The most important and significant discovery which Folin made in these studies was with regard to the constancy of creatinine excretion which varied with the individual but neither with the dietary protein nor with the total N content of urine (17). He considered that in creatinine he had "by far the most reliable index as to the amount of a certain kind of protein metabolism occurring daily in any given individual." Thus arose the Folin concept of N metabolism being made up of an endogenous and an exogenous component. Folin also pointed out that endogenous metabolism was related to body weight. This was confirmed by later workers who also found that it was correlated to the basal heat production (8, 14, 36, 45).

Martin and Robison (30) suggested on the basis of the observed time lag between the change in the level of protein intake and the establishment of N equilibrium that when the intake was high, protein appeared to be stored in the body, the stores being drawn upon during periods of low protein intake. When a person is placed on a low protein or a protein-free diet the rate of loss of protein from the body is determined by the previous dietary history with respect to protein intake. Under normal circumstances a subject on a protein-free diet would reach a low and steady level of urinary N excretion within 8 to 9 days. However, if the protein intake before the protein-free period was not much above the level of minimum requirement or if the dietary protein was of inferior nutritive value, the endogenous level could be reached much earlier, in fact, even in 3 days as was observed by Murlin *et al.* (36). The changes in urinary N excretion following on variations in dietary protein intake are, as had been pointed out by Folin, principally in urea N and not so much in the other nitrogenous constituents. This would seem to be true irrespective of whether the protein was of vegetable or animal origin, urea N in urine being a function of total urinary N as expressed by the equation $Y = -1.5980 + 0.971\,X$ where X is total urinary N and Y the urea N (38). The relationship indicates that even at the lowest levels of urinary N excretion, which are capable of being reached, a substantial proportion of total urine N would be made up of urea N. The latter would obviously be derived from endogenous protein metabolism. Thus there would appear to be no clear demarcation between endogenous and exogenous protein metabolism.

The concept of nitrogen metabolism as consisting of two independent phenomena made up of endogenous and exogenous components was first seriously questioned by Borsook followed by Schoenheimer, Rittenberg and others. The Folin concept implied the turnover of only a small and constant amount of tissue protein represented by creatinine excretion whereas the bulk of the body protein was considered metabolically in an inert state. Further, only a small part of dietary nitrogen was supposed to be involved in protein synthesis for the purposes of replacement of tissue wear and tear, the major portion being catabolised and excreted in urine. Borsook and

Keighley (6) suggested on the other hand that all the protein in tissues was in a dynamic state undergoing continuous breakdown and resynthesis. This did not envisage the 'wear and tear' phenomenon as being distinct from the general protein metabolism in the body. The amino acids derived from dietary proteins together with the amino acids released in tissue breakdown participated in *a*) maintaining the free amino acids in plasma and within the cells and *b*) in tissue protein synthesis, the excess being catabolised and excreted. In this way, the possibility of protein from one tissue supplying amino acids for the synthesis of protein in other tissues could be envisaged (5). The new hypothesis received support from the observations of Madden and Whipple (28) on the regeneration of plasma proteins in dogs subjected to plasmapheresis to be discussed later.

The work of Schoenheimer and associates on protein metabolism with the use of amino acids labeled with isotopic nitrogen lent further support to the new concept. They considered that for an animal in nitrogen equilibrium the continuous processes of breakdown and synthesis must be so regulated by some unknown mechanism as to avoid changes in the total amount of tissue proteins. No distinction between endogenous and exogenous nitrogen metabolism is made in Schoenheimer's theory of protein metabolism. It was even argued that conclusions drawn about the maintenance requirements of protein on the basis of the endogenous output of nitrogen were based on concepts which had no biological reality.

Mitchell (32) agreed that the original concept of Folin did require modification in the light of the newer knowledge but he did not consider Schoenheimer's findings inconsistent with the Folin theory, for Schoenheimer acknowledges that creatine which is being continuously synthesized is not further broken down. It is excreted as creatinine. In that respect, creatinine metabolism would stand apart from the rest of protein metabolism. The fact that phosphocreatine is the precursor of creatinine (5) and that the constancy of the latter is a reflection of the constancy with which phosphocreatine level in muscle is maintained would not invalidate Mitchell's argument. On the other hand, it illustrates the constancy and irreversibility of an 'initial step of degradation' which Mitchell postulates as a common feature of certain catabolic reactions which together make up the endogenous nitrogen metabolism.

The older Folin concept has proved of immense practical value. Although one has come to realise that a complete dichotomy of endogenous and exogenous protein metabolism is untenable, the fact remains that the endogenous metabolism represents a minimum constant component of the total protein metabolism unaffected by dietary levels of protein and the state of protein reserves. This constancy of endogenous metabolism has been utilised successfully to determine the minimum requirements of protein and essential amino acids in human subjects as well as for the determinations of biological value and digestibility of dietary proteins, in spite of the fact that the procedure is considered to be theoretically unsound. Mitchell (34) has recently defended vigorously the validity of Folin's theory and the contribution which it has been instrumental in making to our knowledge in the field of protein nutrition.

RETENTION OF DIETARY NITROGEN

When the intake of protein in an adult exceeds the minimum requirement, some of the excess nitrogen is stored within the body presumably to replenish and add to the protein reserves. A part of this N retention roughly amounting to 1 g per day on an average could be accounted for by the requirements of what Mitchell (33) terms 'adult growth'. The retention of nitrogen can, however, be in excess of these requirements. Cuthbertson, McCutcheon and Munro (11) showed that when additional protein in the form of milk, beef, or soya protein was given to adults in N equilibrium on normal self-chosen diets, about 54% of the excess nitrogen was retained. The stored N could be accounted for by increase in body weight in accordance with Rubner's calculation of 33 g tissue laid down for 1 g N retained.

However, the fact that retention of N in healthy adults is not always accompanied by increase in body weight was demonstrated by Patwardhan and associates (25, 37), who observed continuous retention of 1 to 5 g N/day on diets providing protein within a range of 1 to 1.6 g/kg body weight. Later Bray (7) reported a similar phenomenon, this time in malnourished African children in the Gambia, West Africa. Wallace (51) refers to the work of Benjamin who as early as 1914 was puzzled by the "inexplicable finding of a gain of protein not represented by an appropriate gain of body weight" in normal infants. Whereas this could partly be explained by changes in body composition occurring in infancy and early childhood, the same explanation cannot apply to adults in whom nitrogen retention without weight increase has been observed.

Holmes, Jones and Stanier (22) whose observations were fairly prolonged, extending in some cases for over a year, found at times a retention in malnourished African adults of the order of 10 g N/day on high protein and high calorie diets. Gains in weight were negligible. The authors took into account the fact that changes in body composition such as the loss of body fat and of cell solids, possibly also of bone minerals, and increases in extracellular and intracellular fluids do occur in patients suffering from protein and calorie undernutrition. In the early stages of a high protein dietary regimen, the retained nitrogen helps in building up cell solids and deposition of fat which is accompanied by reduction in extra and intracellular fluid. The two phenomena working in opposite directions will mask the increase in body weight which is expected to occur during rehabilitation. Holmes *et al.*, however, found that N retention without increase in body weight occurred even after the rehabilitation was complete as judged by the return of extracellular and intracellular fluids to the normal levels

The authors did not feel that the cutaneous loss of nitrogen which would ordinarily be of the order of 0.4 g per day (through insensible perspiration only) could materially account for the N retention observed over prolonged periods. It would appear, therefore, that no satisfactory explanation has been forthcoming for this peculiar phenomenon which militates against the assumption underlying Rubner's calculation of 33 g new tissue laid down for every gram of nitrogen retained. Cuthbertson and Munro (12) in their studies on the protein sparing action of carbohydrates and fats had observed that Rubner's concept did not hold good when N retention occurred on higher than normal calorie intakes brought about by non-protein supplements.

Wallace (51) has pointed out difficulties in reconciling the observations on nitrogen retentions to body composition. He points out that, except in the early years of life when body composition is maturing, the concentration of nitrogen in the fat-free body remains relatively constant in spite of differences in the previous dietary protein intake and changes in body weight. The changes occurring in fat and mineral content of the body are too small markedly to influence the interpretation of nitrogen balances. He has also pointed out certain sources of error in the techniques and assumptions in the calculation of nitrogen balances which merit consideration and further investigation.

PROTEIN RESERVES

The capacity to store nitrogen in the body raises some questions pertinent to the nature of protein reserve, its size, the manner in which it is held in the body, whether it consists of several components differing in their availability, and the limit to which these can be drawn upon commensurate with health. Most of these questions are without satisfactory answers.

It has been established that protein reserves are rapidly depleted when an animal is placed on a protein-free diet. The rate at which this happens is determined by the size of the stores. Greater amounts of N are lost through urine when protein reserves are high than when they are low (3, 36). Allison and Wannemacher (4) conclude from their observations on dogs that the replenishment of these stores is dependent upon the quality of protein. That these findings might be applicable to man appears probable from the work of Murlin *et al.* (36) and Cuthbertson *et al.* (11). Forsyth, Shipman and Plough (18), who studied the relation of N retention to N intake in convalescing patients in the anabolic phase, found a linear relationship between the two. When the N intake varied between 0.16 g and 0.86 g per kg on a calorically adequate diet, the relationship could be expressed by the equation, N retained in g/kg. $= 0.016 + 0.19 \times$ N intake in g/kg. They also observed that the rate of nitrogen storage did not appear to be related to the degree of malnutrition.

When a subject who has been placed on a protein-free, diet reaches the level of endogenous urinary N excretion it is generally assumed that the stage has been reached where protein reserves can be considered to have been exhausted. That this is probably not the case was demonstrated by Deuel *et al.* (14). One subject on a protein free diet investigated by them for 30 days had reached the endogenous level of urinary N excretion much earlier. At this stage thyroxine was administered daily for a few days as a result of which a further 141 g N was lost in urine under its influence without affecting the excretion of creatinine and uric acid.

A different approach has yielded useful information on the nature of reserve protein. Pollack and Bookman (39) found excessive amounts of riboflavin excreted in urine in post-operative periods when patients were in a negative N balance. They also observed a similar phenomenon in diabetics who went into negative N balance after the withdrawal of insulin. When N balances became positive during convalescence of surgical patients and in diabetics given insulin, the urinary excretion of riboflavin also decreased and returned to normal levels. In their normal subjects also, these authors observed a higher excretion of riboflavin associated with negative N balance. The authors postulate that since most of the riboflavin in the body exists in combination with protein, the association of excessive excretion of riboflavin with negative N balance implies that labile protein stores which may consist largely of flavoproteins are the first to be drawn upon in protein deficiency or in conditions where protein catabolism is increased. The recent observations of Smith *et al.* (44) in normal human subjects relating to the level of protein intake and riboflavin excretion in urine lend support to this view. Pollack and Bookman further suggested that labile proteins contribute to the metabolic pool of amino acids and other protein metabolites from which stable protein reserves could be built at a slow rate. The work of Deuel *et al.* (14) referred to earlier had indicated the existence of labile and less readily available components of protein reserves.

Flavoproteins are concerned in enzyme action. It is, therefore, to be expected that in protein depletion several enzyme systems would suffer in consequence, even those which are not linked with flavoproteins may be affected. Apart from the numerous observations on experimental animals in which this phenomenon has been demonstrated, its occurrence in clinical protein malnutrition (13, 42, 49) is also well known. Some of the enzyme proteins in tissues may, therefore, be considered to represent a part of labile protein contained in them.

The rate at which body protein is lost and the contribution which different tissues make to this loss have been determined in experimental animals. Addis, Poo and Lew (1) found that body protein losses in 7-day starved rats when expressed in terms of total N loss were as follows: muscle, skin and skeleton 62%; liver 16%; alimentary tract, pancreas and spleen 14%; drawn blood 6%; kidneys 1% and heart 0.5%. When these values were expressed in terms of the original tissue protein the estimated losses were as follows: liver 40%; prostate and

seminal vesicles 29%; alimentary tract, pancreas and spleen 28%; kidney 20%; drawn blood 20%; heart 18%; muscle skin and skeleton 8%. According to Kosterlitz and Campbell (26) it is the cytoplasmic protein including ribonucleoprotein which is readily lost from certain tissues in starvation or on a protein-free diet. The loss of DNA is minimal or negligible. It is thus likely that the labile protein reserves may have different composition in different tissues and may also be lost under stress at different rates (50, 52). However, not much information is available on the size of labile rseserves nor would there seem to be any clear demarcation between the labile reserves and the comparatively stable protein components of the body.

Harroun, Smyth and Levey (21) have used an ingenious method to detect the presence or absence of tissue stores of protein. They determined plasma volume and plasma proteins in 7 normal and 8 undernourished subjects before and 30 minutes after the intravenous infusion of 1 litre of physiological saline solution. They found that in the normal subjects there was an average increase of 441 cc in plasma volume and 13.9 g in circulating plasma proteins. In undernourished subjects the average increase in plasma volume was 170 cc, whereas the total circulating plasma proteins were decreased by 14.7 gm. Harroun *et al.* believed that the increase in circulating plasma proteins observed in the normals was derived from tissue stores and that in undernutrition where protein stores had been exhausted proteins left the plasma and entered the tissues. Albumin contributed most to these changes in either direction. The authors made repeat observations on 2 undernourished subjects during treatment and found a reversal of the trend seen in undernutrition to occur with clinical improvement under treatment.

It can be argued whether it is at all necessary to consider tissue protein as made up of labile reserves and a stable component. As will be mentioned a little later isotopic investigations have shown that proteins from different tissues exhibit different turnover rates. It is possible that proteins with the quickest turnover rates are the first to be catabolised in protein deficiency or starvation. Borsook and Dubnoff (5) suggested that 'labile' proteins need not be different in composition from the stable component. It might merely be a question of difference in the rates of turnover in different locations. Proteins from liver, intestine and kidney seem to be the first to be affected and are largely lost in the early stages of starvation or protein depletion. Their replenishment would be limited by the availability of amino acids released by the breakdown of protein with slower turnover rates as in muscle. Under these conditions, tissues such as liver, intestine, etc., may continue to subsist with a minimum of cytoplasmic protein which is usually and probably wrongly termed the stable component.

NITROGEN POOL AND PROTEIN TURNOVER

The researches of Borsook, Whipple, Schoenheimer and their associates led to the concept of a common metabolic pool from which amino acids are available for the synthesis of proteins in various tissues and organs. The metabolic pool was defined by Sprinson and Rittenberg (46) "as that mixture of compounds derived either from the diet or from the breakdown of tissues which the animal (or organ or cell) employs for the synthesis of tissue constituents. The nitrogenous compounds of the metabolic pool constitute the nitrogen pool." Urea, creatinine and uric acid being purely excretory products do not form a part of this pool. However, this categorical statement on urea may require some modification in view of the observations of Moore, Lavietes, Wakeman and Peters (35) who had found daily retention of 1.9 to 3.5 g N in human subjects fed 35 to 42 g urea for 3 to 19 days. According to Rose and Wixom (40) and Holt and Snyderman (23), urea can be utilised for maintenance in adults and growth in infants when it is given as a source of non-essential nitrogen to supplement essential amino acids. Widdowson (54) further suggested that urea may be utilised in protein-depleted subjects for purposes of tissue protein synthesis.

Information on metabolic pool, protein pool, rate of protein synthesis and degradation, nitrogen exchanges in the body etc., has been obtained with the use of amino acids or proteins isotopically labelled with N^{15}, C^{14} or I^{131}.

Sprinson and Rittenberg (46) estimated that in an individual in N equilibrium subsisting on normal protein diets and administered glycine-N^{15}, nearly 70% of the latter was used in tissue protein synthesis. Less glycine-N^{15} was utilised in this manner in subjects on high protein intakes, a larger proportion being metabolised and excreted in urine. The authors utilised the N^{15} excretion studies to estimate *a*) the daily synthesis of tissue proteins, *b*) the size of the metabolic pool, and *c*) the turnover rate of tissue protein. According to them 0.199 g. N/kg body weight was used up daily for tissue protein synthesis, the size of the metabolic pool being estimated as 0.514 g N/kg body weight. The average half life of tissue protein was calculated at 80 days. The turnover rate of protein in different organs in the body was, however, different, e.g. the estimate for the half life of protein in liver and blood plasma was 10 days and in spleen, heart, kidneys, small intestines, pancreas and endocrine glands 20 days. In muscle, the protein turnover rate was estimated to be the slowest with a half life of 158 days. The calculations of Sprinson and Rittenberg show that a little over half of the protein daily synthesized was accounted for by liver, spleen, heart, kidneys, small intestines, pancreas and endocrine glands.

In 1953, San Pietro and Rittenberg (41) revised the earlier estimates for the size of the metabolic N pool which were considered too high for they were likely to include the urea pool. According to them, the metabolic pool is made up of the total free amino acids in the body which was estimated for their 2 subjects to be of the order of 2.2 to 2.3 g N on the basis of 6.5 mg amino acid N/100 cc blood and 50% of the body weight as total body water. Crispell, Parson and Hollifield (10) using N^{15} glycine give for 5 normal subjects, values for

the metabolic pool between 10 to 17 mg. N/kg, which are even lower than those of San Pietro and Rittenberg. The argument that the metabolic N pool should consist of only free amino acids seems to be at variance with the definition of the pool given earlier by Sprinson and Rittenberg according to which all nitrogenous constituents derived from protein and capable of being utilised for protein synthesis would constitute the pool. The estimates of San Pietro and Rittenberg and Crispell *et al.* obviously refer only to the metabolic pool of free amino acids in the body. Sharp *et al.* (43) using N^{15} tagged yeast protein gave the estimates for the size of the metabolic N pool as between 0.45 to 0.70 g N/kg body weight in 6 subjects observed by them. These values are similar to those reported by Sprinson and Rittenberg. The rates of protein synthesis estimated by Sprinson and Rittenberg and by Sharp *et al.* are much lower than the figure reported by San Pietro and Rittenberg which is comparable to the rate of synthesis of 756 mg N/kg/24 hr. (average) for 5 healthy subjects reported by Crispell *et al.* (10). It appears as if uncertainty does exist with regard to the size of the metabolic N pool and the rates of protein synthesis in man.

Evidence pertaining to the influence of undernutrition on the turnover rates of proteins is scanty. The findings of Steinbock and Tarver (47) in rats indicate that replacement rate of protein is considerably diminished in protein deficiency. On the other hand Hubay *et al.* (24) have found no significant differences in the rate of disappearance from plasma of I^{131} labeled plasma protein in protein-depleted dogs and hypoproteinaemic human subjects. Eckhardt and Davidson (15) had observed that mere undernutrition (in man) did not alter the rate of metabolism of intravenously administered serum albumin. Madden (27) also finds no difference in *a*) equilibration time between the intra- and extravascular compartments, *b*) the size of the albumin pool and *c*) total exchangeable albumin, in 3 cachectic patients in negative N balance even after they were adequately fed and showed large nitrogen balances. Further information on this subject is needed.

The protein pool is distinct from the metabolic pool which according to some authors consists presumably of free amino acids. The two, however, are closely interrelated and in dynamic equilibrium with the plasma-free amino acids (31). There need not be a single protein pool serving all the tissues. The fact that proteins in different tissues have different turnover rates should support the argument that each tissue has its own intracellular protein pool. Uncertainty exists with regard to the size of the protein pool. Observations with I^{131} labeled human serum albumin have shown that the total exchangeable serum albumin is somewhat greater than twice that circulating in the blood (9, 48). Comparable information for other tissues is not available. Even for serum albumin where the pool size and turnover rates have been determined, there is confusion with regard to the latter owing to a variety of isotopically labeled compounds used. Margen and Tarver (29) estimated serum albumin turnover by using S^{35} labeled albumin, I^{131} labeled albumin and S^{35}-cystine or methionine. The longest (43–46 days) half life was found with S^{35}-cystine and the shortest (15 days) with I^{131}-albumin.

METABOLISM OF PARENTERALLY ADMINISTERED PROTEIN

The concept of the dynamic state of tissue protein, metabolic N pool and protein reserves had arisen independently, as mentioned earlier, out of the observations of Whipple and associates who used dogs for studies on plasma protein regeneration after subjecting them to plasmapheresis. It was found that healthy dogs on an adequate diet could maintain the normal plasma protein level in spite of repeated plasmapheresis resulting in the removal of as much as 25% of the total blood plasma. When such a dog was subjected to fasting it formed plasma proteins from tissue proteins in an attempt to maintain the plasma protein level; when starvation was followed by placing the dog on a limited diet, a steady formation of plasma proteins from tissue proteins could be demonstrated. The authors concluded that there were reserves of protein in tissues which contributed to the maintenance of plasma protein concentration in fasting or during dietary protein deficiency.

Dietary proteins differed in their capacity to regenerate plasma proteins. Parenterally administered proteins or amino acid mixtures were also shown to contribute to the formation of plasma proteins in addition to maintaining the other protein needs of the body. The important conclusions arising out of the classical work of Whipple (53) and his school can be summarised as follows: *1*) Proteins from tissues are raided to provide plasma proteins. The latter are reformed when removed and exceed in quantity the dietary protein supply under conditions of protein restriction. *2*) Parenterally administered plasma can be utilised for maintaining N balance and the normal level of plasma proteins in dogs with minimal weight loss. *3*) Parenterally administered C^{14} labelled plasma proteins are rapidly incorporated in tissues. In 7 days, 62% of administered C^{14} is recoverable from tissues. The rest can be almost totally accounted for by plasma (24.6%), expired air (4.9%), RBC (4.3%) and urine (1.3%). The minimal losses of radioactivity in expired CO_2 and in urine have been interpreted to suggest that parenterally administered protein is delivered intact to tissues where it possibly is degraded to peptide state—and not to amino acids—and incorporated in tissue proteins. If that were not so more radioactivity should have appeared in urine and expired CO_2.

The researches of Whipple had a significant influence on the development of the technique of parenteral protein nutrition in man. This technique incidentally provided a tool for the elucidation of problems connected with the interrelation between plasma proteins and tissue proteins and the mode of utilisation of parenterally administered protein within the body. Attempts in this direction, particularly where the latter aspect is concerned, have not however been fully successful as will be evident from the following discussion.

Albright *et al.* (2) administered plasma proteins intravenously to normal subjects and hypoproteinaemic patients and observed that in both the administered protein was catabolised at a slow rate. They further studied the changes in urinary nitrogen, phosphorus and potassium occurring after the intravenous administration of protein and utilised this information to compute the relative proportions of the administered protein which were catabolised and converted into tissue protein. They concluded that at a certain stage, i.e. about the eighth post-injection day, the ratio of protein 'burnt' to protein 'converted' was 1:1 and that these two accounted for the entire administered protein. Thereafter the catabolism of 'converted' protein become predominant. Eckhardt and Davidson (15), who administered human serum albumin to normal subjects and malnourished patients, estimated that in normal subjects about two fifths of the intravenously administered serum albumin was converted into tissue proteins, whereas in malnourished cases almost the whole of it could be so utilised. The level of protein in plasma was increased and the extravascular compartment was enriched increasing the concentration of proteins in the fluid bathing the cells. Blood protein level returned to normal in about 2 weeks. A slow breakdown presumably at the cell surface released amino acids which could either be used up for protein synthesis within the cell or deaminated, oxidised and excreted in urine. A gradual disappearance of protein from the blood and a stepwise increase in urinary N were considered as evidence in support of the above suggestions.

Gimbel, Riegel and Glenn (20) studied N metabolism in 3 healthy adults given human serum albumin intravenously as the sole source of nitrogen. They confirmed that intravenously administered albumin disappeared from the plasma slowly and accumulated in plasma, interstitial fluid and lymph. They also observed a positive N balance in the early stages, N equilibrium being reached in 14 to 20 days. Both the administered albumin and the protein-free diet contained very small quantities of potassium. The retention of potassium observed during the period of positive N balance was considered as indicative of tissue protein synthesis. A protein-like globin prepared from haemoglobin, on the other hand, when given intravenously on a calorically adequate diet is rapidly metabolised and is also excreted unchanged (about 25%) as indicated by marked proteinuria in the first 24 hours after injection (19). This must be due to the incompleteness of the protein characterised by the marked deficiency of isoleucine. Denatured protein is also catabolised rapidly when given by the intravenous route (29).

There is thus a marked difference between the utilisation of protein fed orally and given intravenously. In the latter case the utilisation is much slower since the bulk of the protein is held for some time in the intra- and extravascular compartments and is only slowly broken down as seen by the delay in the increase of urinary N as compared to that obtained with orally fed protein. Amino acids (as mixtures of pure amino acids or from protein hydrolysates) on the other hand are equally rapidly metabolised when administered by either of the routes. When protein needs of the body are to be met by parenteral therapy, intravenous protein has the advantage over amino acids, on account of the slower metabolism and probably more efficient utilisation of the former (15). Protein under these conditions is retained in the body and utilised for tissue protein synthesis. It was held at one time that the retained protein passed into the cells where it was broken down and amino acids reutilised. McFarlane (31) in reviewing the available evidence considers this as improbable. Protein which disappears from the plasma accumulates in the extravascular compartment such as lymph. It is broken down mostly at cell surfaces and to a small extent within the cell, and the amino acids are almost completely reutilised for tissue protein synthesis.

The question whether parenterally administered proteins are broken down to peptide or amino acid stage before incorporation into tissue protein is one on which no conclusive evidence is forthcoming even from animal experiments. It is not proposed to venture into a discussion of this subject for it is outside the scope of this article.

CONCLUSION

The question of protein reserve is of utmost importance in human nutrition. Minimum intake of protein which is needed to replace the protein lost by endogenous metabolism does not permit the building up of protein reserve. It is also argued that in the absence of such reserves the body is unable to cope with the demands made by infections and injury. Protein reserve is therefore considered necessary for optimum protein nutrition. The resolution of the difference between the optimum and minimum protein requirements will depend upon our obtaining reliable information on the few problems discussed in the preceding pages.

The significance of nitrogen retention when not accompanied by body weight increase, if understood, will possibly throw some light on the nature of protein reserve. Independent methods for measuring protein reserve are also necessary. Although it has to be admitted that the knowledge gained from isotope experiments must preclude a rigid differentiation between labile reserve and stable tissue protein, the fact that a part of protein is readily lost from some tissues in the body should not invalidate the assumption that the labile and stable components may probably differ in composition and possess different properties signified by the lability of one and the stability of the other. Differences in the composition of protein in various cell fractions should make it clear that one is confronted with different proteins within a cell with different functions and possibly with different turnover rates. Similarly an insight into the rates of protein synthesis in

health and as influenced by protein depletion with or without calorie undernutrition will assist a great deal in practical measures of rehabilitation of malnourished individuals. That all the above problems are interrelated and need elucidation is a fact which it is needless to emphasize.

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Vegetable protein mixtures for human consumption[1]

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THE WIDE-SPREAD PREVALENCE and great public health significance of protein malnutrition among young children in most technically underdeveloped areas of the world is now well recognized. Educational measures are generally of value, but successful prevention often depends on the availability of sources of good quality protein. While it is frequently possible to increase production and consumption of protein-containing foods of animal origin such as milk, cheese, meat, eggs and fish, these foods are often beyond the economic reach of the population segments needing them most. In many countries, it is impracticable to provide all the foods of animal origin necessary to furnish the major part of protein requirements. Furthermore, this situation tends to become worse as populations increase. Better use of vegetable protein is the only practical solution to the problem of protein malnutrition for many areas.

Since individual plant proteins are relatively poor in biological value because their amino acid content does not suit mammalian needs, they must either be supplemented with animal protein or mixed with other plant proteins which have a complementary amino acid content. The other alternative of supplementing with synthetic amino acids may some day be practicable, but costs are still prohibitive. The first mentioned possibility, combining animal and vegetable protein sources, is the basis of the ordinary good mixed diet. When skim milk, fish flour and certain other animal products are available at relatively low cost, they may, of course, also be of major importance in technically underdeveloped areas. The primary purpose of this paper, however, is to describe the progress which has been made in devising vegetable combinations of low cost and high protein value.

BASIC PRINCIPLES INVOLVED

In recommending the supplementation of predominately vegetable diets with proteins of animal origin, the greater the proportion of animal to vegetable protein, the higher the nutritive value, although beyond a certain point there is no practical advantage. When adding protein-rich foods of vegetable origin to a diet, however, there is an optimum proportion above or below which biological value is decreased. For this reason, the combination of proteins of vegetable origin for child feeding, where protein quality may be critical, requires a degree of precision which implies the elaboration of a mixture available as a single protein source as nearly comparable as possible in quality to protein-rich foods of animal origin.

It is obvious that in addition to having a high protein quality, such a mixture should be a relatively concentrated source of protein, at least before preparation as a beverage or mixing into other components of the diet. Preliminary estimates of the protein quality of a vegetable mixture may be made from a comparison of the amino acid composition with a theoretical amino acid pattern such as that of the FAO Reference Protein (21), or a good animal protein such as supplied by egg or milk.

There are several reasons, however, why a protein mixture may be better or worse than such a comparison would suggest. One reason is that current amino acid assay procedures give the amount present but not the amount available to the human organism. Furthermore, the amino acid proportions given in the FAO Reference Protein appear too high for methionine and tryptophan, and too low for lysine (7, 8, 33), and no other theoretical pattern has been proved to be any better. A relative amino acid imbalance may also interfere with the utilization of individual essential amino acids, rendering the protein score unreliable. The final test thus becomes the protein value as determined in suitable experimental animals together with effectiveness in child feeding trials. In INCAP, we find the comparison of the nitrogen retention of children fed various vegetable mixtures with that of children receiving isoproteic amounts of milk, a particularly useful means of evaluation.

In devising protein-rich vegetable mixtures, it is important to specify the objective and form of use. Protein malnutrition is most acute in children 1–5 years of age who, after weaning, are not given a diet with adequate protein. This age group needs a supplement to their customary diet which will make up the protein deficit and also correct any other nutrient deficiencies. This means that for most areas, the mixture should be a good source of vitamin A, riboflavin and calcium, and preferably that it also supply supplementary niacin. Iron may also be needed. In Latin America, at least, adequate amounts of thiamin and vitamin C are usually supplied by the cereals and fruits common in the diets. The desirable caloric content of the supplementary

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food depends upon the adequacy of the habitual diet, and is usually of minor concern.

When they are fed as a semisolid, vegetable mixtures for the mixed feeding of infants 6–12 months of age, who are still receiving mothers' milk, do not present special problems. If the vegetable mixture is prepared as a liquid, care should be taken, as with supplementary milk feeding, not to displace breast feeding prematurely.

A vegetable mixture formula employed for infant feeding when neither breast milk nor cow's milk is available, carries with it additional problems. It must be recognized that a vegetable mixture may be entirely satisfactory for the prevention of protein malnutrition in older children and quite unsatisfactory as the basis for the formula feeding of young infants. The suitability of a vegetable mixture for feeding premature infants has still less practical importance if its primary purpose is the prevention of protein malnutrition in preschool children. The use of vegetable mixtures as supplementary sources of protein is, of course, not limited to children. It is also beneficial for pregnant and lactating women, and acceptance as a staple in the diet of all members of the family, increases the probability that the mixture will be made available to the family members who need it most.

In developing vegetable mixtures it is important to consider agricultural, economic and cultural factors simultaneously with nutritional value and palatability. There are probably many vegetable mixtures of high nutritive value which can be produced from the vegetable resources of most areas, but most of these will prove to be unacceptable or too expensive if nutritional value is the only major consideration.

PRESENT SCOPE OF EFFORTS TO DEVELOP VEGETABLE PROTEIN MIXTURES

Under the stimulus of WHO and FAO, numerous international nutrition and health conferences in the last decade have stressed the need for developing new sources of protein-rich foods for human feeding. The joint WHO-FAO-Macy Foundation Conference on Human Protein Requirements held in Princeton in 1955 (44) led to the designation by WHO of a Protein Advisory Group to give international guidance and encouragement to programs designed to develop new protein sources. It also resulted in a substantial research grant from the Rockefeller Foundation to be administered by a committee of the National Research Council for the same purpose. Personnel of FAO and UNICEF, as well as WHO, have participated actively in these programs. Projects sponsored by the NRC Committee are now underway in Mexico, Guatemala, French West Africa, Nigeria, Belgian Congo, Uganda, Union of South Africa, India, Japan, United States, United Kingdom, Taiwan and Brazil, and many additional centers are engaged in this work with other support. Not all of these projects have as yet produced results, and some deal only with the use of animal protein to enrich plant protein mixtures or are basic biochemical and biological studies of plant proteins. It is obvious, however, that with such interest in the problem and so many centers actively engaged, rapid progress can be expected in this field.

VEGETABLE INGREDIENTS FOR PROTEIN-RICH FOODS

From a practical point of view the elaboration of protein-rich vegetable mixtures involves the supplementation of the cereal grains which form the staple food in most technically underdeveloped areas. Unfortunately, all cereal grains have the double disadvantage of a relatively low protein content which often decreases further with processing, and a poor protein quality due to deficiencies of several of the essential amino acids. These disadvantages can be corrected by combining cereal grains with foods of higher protein concentration which supply essential amino acids in such proportions as to correct the deficiencies in the cereal grain.

Five large groups of vegetable products which could be used for this purpose are: *1*) legume seeds, *2*) oil seed cakes, *3*) nuts, *4*) palm kernels, and *5*) leaf proteins. Of these, some have received attention as protein supplements, but many have been neglected or require further study. Although algae and yeast have frequently been suggested as protein sources, thus far, they have not proved sufficiently palatable to constitute more than a small percentage of the total diet.

The legume seeds form a vast group with a protein content of around 25%. In general, their quality as single protein sources is not much better than the cereal grains. They are, however, relatively good sources of lysine, the first limiting amino acid in cereal grain protein, but are limiting in methionine, which is adequate in cereal grains. Legume seeds must be cooked for maximum utilization, and improper processing may decrease their protein value. Actual protein value varies widely within the group which includes the peanut, *Arachis hypogaea*, the soybean, *Glycine max*, the common bean, *Phaseolus vulgaris*, the cow pea, *Vigna sinensis*, the chick pea (Bengal gram), *Cicer avientinum*, and beans of the genera *Vicia*, *Pism*, *Lens*, *Dolichos* and *Canavalia*. *Phaseolus vulgaris*, *Glycine max*, *Pism sativum*, and *Arachis hypogaea* seeds have been studied, but more research is needed on these and the other genera if full advantage is to be taken of their potential contribution to the prevention of human protein malnutrition.

Since soybean has been a human food for centuries in certain parts of the world, and because it is now an important commercial source of oil and of press cake for animal feeding, this legume has received the greatest attention. It has been prepared in a variety of forms for human consumption and the literature is voluminous (15, 38). Cooked soybean meal contains approximately 44% protein of a quality superior to most vegetable proteins but inferior to most proteins of animal origin. Its strong, slightly bitter flavor has limited its acceptability in some populations.

Of major current interest are the efforts to develop soy protein isolates of more concentrated protein content

(90%) (36), and to adapt fermentation procedures used on a home-scale in the Far East (2, 16) to commercial production. The peanut or groundnut has been widely investigated because the commercial value of its oil contributes to the low cost of the meal. Like other legume seeds, peanut is limiting in methionine and relatively low in arginine, lysine and isoleucine compared to animal protein (31).

The second group of important protein sources is that of oil seeds such as cotton, sesame, sunflower, rape, mustard, poppy, linseed, safflower, hemp and several others. Many of these are produced in such small quantities that they have no practical importance at the present time, and several have specific disadvantages of flavors or toxic substances which limit their usefulness. Because cotton is produced extensively for fiber, cottonseed is readily available for oil extraction, and cottonseed presscake is produced at low cost in such large quantities that it is of major importance as a protein concentrate in animal feeds. It has the disadvantage of containing gossypol which is toxic to some animals including the guinea pig, hampster, pig and rabbit, and which reacts to render lysine unavailable to the animal organism (1). Technological developments, however, have resulted in the production of cottonseed flours with protein of high nutritive value and sufficiently low free and total gossypol concentration that they can be fed liberally even to the most sensitive animals (1). It is probable that nearly gossypol-free flours of higher protein concentration and better protein quality will be developed. At present, good cottonseed flours for human consumption are low in both total and free gossypol and have a protein content in excess of 50% with a biological value of about 65%. As will be discussed in another section, they have a very favorable amino acid pattern for complementing cereal proteins.

Sesame has a nutritive value similar to cottonseed. It has no known toxic principle, but the removal of the hard hull is necessary to reduce the crude fiber content and to eliminate a slight bitter aftertaste. It is not popular with agriculturists because the seed pod of most varieties complicates harvesting operations by shattering readily. New varieties are being developed to overcome this difficulty. Sunflower seed is another good source of both oil and protein concentrate, but so far its large-scale production is limited to Argentina, Chile and Russia. The possibility of using the other oil seeds has not yet been investigated.

The true nuts are not sufficiently plentiful or low in cost to offer important practical advantages for large scale protein supplementation of cereal diets. Of the palm kernel proteins, only copra has been studied. A review on copra protein by Curtin (12) discusses production, composition, processing and properties. Lyman (29) has indicated that lysine is probably the first limiting amino acid, while work on protein isolates of coconut has shown that some of these fractions are good sources of lysine but low in tryptophan (9). Copra protein has been used in low cost protein foods in combinations with peanut flour and Bengal Gram (28). The results in experimental animals were satisfactory and acceptability trials gave excellent results. A disadvantage of copra protein is its high fiber content. Little is known of the other palm nut meals, but their crude fiber content is equally unfavorable. If the demand were great and the nutritive value of the protein sufficiently high, procedures for the removal of part of the crude fiber and preparation for human consumption could be developed.

Methods for the separation and concentration of leaf protein have recently received considerable attention (16, 30). At the present time, however, the cost is high and the amount which is acceptable in human diets limited. While these difficulties can probably be overcome, the quality of the protein is no better than that of the legumes, and leaf proteins are not as effective in improving the protein quality of cereal diets because of a relative deficiency in methionine-cystine. Studies of Hughes and Eyles (24–26), and Ellinger (18) in poultry have shown that the gross protein values of leaf protein are generally lower than expected from their amino acid composition, probably due to low digestibility. Nevertheless, improvement of the nutritive value of rice by adding a cooked leaf meal has been reported (40).

Many other basic plant materials might also serve as sources of protein concentrates. The by-products of the cereal industry should be mentioned among these, particularly the germ of the seeds. They are rich in oil and the protein is of good quality. Wheat germ is already an accepted human food; similar uses could be found for the maize germ left from the production of corn starch and for the rice germ which is a by-product of rice milling. Maize germ has been used in South Africa (23) to enrich vegetable mixtures.

SOY BASED PROTEIN-RICH FOODS

Dean (13, 14) was one of the first to propose a soybean-banana mixture for the treatment of kwashiorkor; this he later abandoned in favor of better balanced mixtures. Following Dean's suggestion, Thompson (41, 42) attempted therapy with such a mixture in 42 cases, but only 55% gave satisfactory results. It was difficult to achieve sufficiently high protein intakes because the fluid-soy mixture for tube feeding had 2½ times the volume of skim milk with an equivalent protein content. INCAP abandoned this type of regimen in 1955 when only 1 of 3 kwashiorkor patients so treated responded satisfactorily.

Soybeans must be soaked, skinned, minced and cooked before being fed, which limits their use for infant feeding in the home. Dean (15) reviewed the few reported trials where comparison with milk was adequate and found the results inconclusive, due partly to failure to neutralize the trypsin inhibitor. Moreover, several of the preparations seemed to cause gastrointestinal upsets. The value of soybean protein, even for mixed feeding,

is thus limited by the difficulties of preparation. Subrahmanyan and co-workers (38) reviewed additional evidence that soybean milk can be made an adequate food for infants, but they also concluded that more studies are needed. Work with a fungus treated soybean product tempeh, and with manufactured preparations such as the Indonesian 'saridele' and with U.S. proprietary 'soy milks,' hold promise that the problems inherent in the use of soybean for infant feeding will eventually be solved (2).

Soybean can be processed to increase its percentage of protein and its nutritive value can be raised by adding synthetic amino acids. A product with 14.2% protein supplemented with 240 mg of methionine and 16 mg of cystine per 100 g plus vitamins and carbohydrate has been tested for acceptability by Barnes (3), and for nitrogen retention by Fomon and May (20). No difference was found in the rate of growth or nitrogen retention of infants, whether the entire protein intake was derived from human milk or 41–45% from the soy preparation. In a subsequent study, Fomon (19) gave to 4 infant girls for 36–72 days a formula with 1.14 g of soy protein per 100 ml without amino-acid fortification as the sole source of protein; he obtained growth and nitrogen retention results as good as those with human milk. It should be noted that this was a specially processed multi-ingredient formula, quite different from cooked crushed beans, and that the daily protein intakes averaged 1.7 g/kg of body weight.

Bassir (4) gave 30 g of soy flour daily for 3 months to nursing mothers during the first 6 months of lactation and reported a significantly higher milk output with no change in quality as compared with an equal number of controls. The soy flour supplemented ad libitum diets of meat, bread, rice, beans, cassava and yams.

In an extensive series of studies in rats, Gilbert and Gillman (22) have examined the effectiveness of soy as a supplement to maize protein at levels from 2 to 20% of the protein in the diet by measuring growth, survival and gross organ pathology. Although 'skimmed milk powder complemented in every way the various ingredients,' growth was improved very little by added soybean up to 10% of the protein in the diet even when vitamins A, E and D, yeast and salts were added; with 20% of soy protein the results still fell far short of these with the control diet containing 20% protein from skim milk powder and the remainder from maize. DeMaeyer and Vanderborght (17) found soy flour less effective in increasing nitrogen retention in children when added to a diet of rice, bread, banana flour, butter, palm oil, sugar and fruits, than isonitrogenous amounts of milk, beans and peanuts or peanut flour. The supplements supplied 75% of the nitrogen and 50% by weight of the total diet.

For countries with a large soybean production, soy protein is undoubtedly a valuable addition to the diet. There has been, however, a tendency to focus too much attention on the possibilities of soy for countries not producing it, to the neglect of more immediately available local protein sources. On the other hand, where soy is grown, research should be intensified to assure its most practical and effective utilization for human consumption.

MIXTURES USING CHICK PEA, COW PEA, BEANS, PEANUT FLOUR OR SESAME

The Central Food Technological Institute, Mysore, India, has developed several formulas for an Indian multipurpose food (39). Two of these known as Mysore Food A and B, have undergone extensive biological trials in the Nutrition Research Laboratories in Hyderabad, India. Mysore Food A apparently contains 25% Bengal gram (chick pea) 'dhal' and 75% peanut meal, and Mysore Food B, 25% Bengal gram, 65% peanut flour and 10% sesame meal. Bengal gram was previously shown by Venkatachalam *et al.* (43), to give good results in the treatment of 56 cases of kwashiorkor compared with 49 given skim milk.

In more recent studies in 53 children (27) with kwashiorkor, the administration of Mysore Food A and B gave results as good but no better than previously obtained with Bengal gram alone. Serum albumin regeneration was notably slower with these 3 than with milk. Peanut protein alone did not promote good serum albumin regeneration although it was of some clinical benefit. In these studies the addition of peanut flour to Bengal gram, with or without sesame flour, did not improve serum protein regeneration. When the treatment was sufficiently prolonged or the daily protein intake increased enough, satisfactory levels of serum albumin were achieved with any of these regimens.

Venkatachalam *et al.* (43) have also compared isoproteic diets of Bengal gram and banana, Bengal gram and rice, and wheat bread and skim milk in the treatment of 124 cases of nutritional edema syndrome. The response to the 2 exclusively vegetable protein diets was on the whole satisfactory, and there was no difference among the 3 treatments as regards disappearance of edema, attainment of minimum weight or subsequent weight gain.

The group for research on infantile malnutrition of the Children's Hospital in Mexico City, have investigated the nutritive value of a mixture of lime-treated maize (as tortillas) and black beans in the 4:5 proportions by weight common in some Mexican diets. The slope of the weight gain of rats fed this mixture was only 0.93 ± 0.037 as compared with 1.84 ± 0.040 when 22% of the protein came from milk (1). Similarly, the absorption and retention of nitrogen by malnourished children was variable from child to child and much lower than with isocaloric diets containing isonitrogenous quantities of milk (10). The addition of tryptophan and lysine to the maize-bean diet greatly improved nitrogen absorption and retention in all 4 cases. The obvious challenge is to find an inexpensive local protein source which will supply additional protein and also help correct the tryptophan and lysine deficiency of maize (7, 33), or a mixture of maize and beans as above.

In common with other investigators, Hansen and coworkers (23) found that milk initiated cure of kwashiorkor without difficulty; a 2-component mixture of 66% maize meal and 33% cow pea meal, however, did not initiate cure in the 3 cases treated with it. A 3-component mixture made up of equal parts of maize meal, maize germ and cow pea, did bring about satisfactory recovery in the one case in which it was employed. It should be noted that it would require 238 g of the dry 3-component mixture and 267 g of the 2-component mixture to supply the essential amino acids contained in 100 g of skim milk. Since the vegetable formulas also required relatively greater dilution, it was difficult to feed enough of the vegetable formulas to supply protein needs. We consider excessive bulk for protein content to be one reason for our lack of success in initiating cure in kwashiorkor with mixtures of maize and beans, and this is the same problem encountered with ordinary soy preparations. The differences in biological value of the protein are clearly reflected in the nitrogen retentions which averaged 13–14% for milk, 8.8% for the 2-component mixture and only 5.7% for the 3-component mixture. The percentage of nitrogen absorbed was also much lower with the vegetable mixtures. It was concluded that either the 2 or the 3-component mixture is satisfactory to promote consolidation of cure and to prevent kwashiorkor, but only the 3-component mixture is of sufficiently good concentration and protein quality to be satisfactory for use in treatment.

Sénécal (35) has reported on the use of 4 kinds of supplementation of the diets of children with incipient kwashiorkor seen in an out-patient clinic in Kakar. These were *1*) 60 g of millet flour, *2*) 24.6 g millet flour, 24.6 g peanut press cake, *3*) 23.0 g millet, 23.0 peanut press cake and 4.2 g fish flour, *4*) 46 g of skim milk powder. The first contained 5 g of sugar and the remainder 10 g each. The total protein provided was 4.7, 8.8, 11.9 and 16.6 g, respectively, with slightly over 200 calories in each supplement. Results with *supplements 2* and *3* were much better than with *supplement 1*, but the results with the skim milk were poorer than with *supplements 2* and *3*. Even the millet supplement, *no. 1*, caused some improvement in growth.

The nitrogen balance data furnished by DeMaeyer and Vanderborght (17) on the supplementation of a mixed diet with either skim milk, a combination of beans and peanuts, peanut flour or soybean fed to children convalescing from kwashiorkor, merit further mention. Their basal diet supplied from 11 to 47% of the nitrogen intake from rice, bread and banana flour, and the remainder from the test supplement. Nitrogen absorption and retention were measured for each supplement at intakes varying at intervals of 100 mg of N from 2.0 to 5.8 g protein per kg. Skim milk had the highest supplementary value by a considerable margin and the combination of beans and peanuts came next, followed by soybean flour and peanut flour.

Of great practical importance was the constant relationship between both the nitrogen absorbed and ingested, and the nitrogen retained over the entire range of protein intakes. The slope of the linear regression line for milk of absorbed nitrogen plotted against that retained was 0.78 and had not begun to decrease at the highest level tested. The slopes for beans and peanuts, peanut flour, and soybean were 0.72, 0.65 and 0.62, respectively. These data mean that in terms of protein, somewhat higher levels of the vegetable supplement gave the same total amount of protein retention as lower levels of milk. If vegetable supplements are cheap and available, they could thus be used in larger quantities to get the same protein effect as milk if they were of relatively good quality to begin with and providing they were in a form which permitted consumption in sufficiently large amounts.

INCAP VEGETABLE MIXTURES 8 AND 9

As a result of 7 years of cooperative work between the Instituto Agropecuario Nacional of Guatemala (IAN) and the Institute of Nutrition of Central America and Panama (INCAP), preliminary descriptions were published in 1957 (5, 6, 32, 34) of INCAP Vegetable Mixture 8. Mixture 8 has the following formula: dried maize masa 50%, sesame flour 35%, cottonseed flour 9%, Torula yeast 3% and Kikuyu grass leaf meal 3%. It has 25.1% protein, 13.7% fat and 503 calories per 100 grams. Comparing the amino-acid pattern of Mixture 8 with the FAO Reference Protein, the limiting amino acids in decreasing order are lysine, tryptophan and methionine with scores of 83, 86, and 91, respectively.

The lime-treated maize (34) is the cereal base for the mixture and a source of calcium. The sesame flour, as supplied (American Sesame Products, Inc.), was pressed from the de-hulled seeds and contained 33% fat. The cottonseed flour was a high protein, low gossypol product supplied under the name of 'Pro-flo' (Traders Oil Mill Co.). The Torula yeast provides B-complex vitamins and the carotene-rich leaf meal is added as a source of vitamin A activity.

Basic animal studies leading to this development have been recently reported (6, 37). Extensive trials with rats and chicks showed that the mixture sustained good growth although it was improved by the addition of lysine. It proved to be well tolerated by children and,

TABLE 1. *Formulas for INCAP Vegetable Mixture 9*

Ingredients g/100 g	9	9′	9A	B9
Whole maize				
Lime treated	28			
Uncooked		28		29
Cooked			29	
Sorghum				
Uncooked	28	28		29
Cooked			29	
Cottonseed flour	38	38	38	38
Torula yeast	3	3	3	3
Dehydrated leaf meal	3	3		
$CaCO_3$			1	1
VITAMIN A IU			4500	4500

surprisingly, to give a nitrogen retention equivalent to milk in a series of 5-day balance trials carried out in 5 children fed at levels of 2.4–3.8 g protein per kg of body weight. After children recovering from kwashiorkor had done well on the mixture as the sole source of protein for periods exceeding 2 months, it was used for the treatment of 7 children with acute kwashiorkor. The results were similar to those customarily obtained with milk except for a slightly slower regeneration of serum albumin. From this work there was no doubt that a relatively simple vegetable mixture could be prepared which would be an excellent source of protein as well as other essential nutrients.

When the time came to plan its possible commercial production at low cost, it was discovered that the supply of sesame seed in Central America had decreased and the price had risen. This, coupled with difficulties in harvesting and in separating the husk, make it desirable to search further for an equally nutritious formula which would eliminate the necessity of using sesame at this time. All of the experience with the cottonseed flour in Mixture 8 and in a long series of biological trials in rats and chicks previously carried out in IAN and INCAP had proved favorable. Accordingly, in 1957 a new formula was developed based on cottonseed flour as the principal concentrated protein source. In it, sorghum was optionally substituted for part of the maize to reduce the cost. Preliminary calculations suggested that on the basis of an optimal combination of cottonseed flour and cereal grain, this should give a mixture of slightly higher protein content and slightly lower protein score if the Torula yeast and leaf meal were held at the same concentration.

As shown in table 1, the formula for INCAP Vegetable Mixture 9 went through a number of variations, the original formula used lime-treated maize and sorghum grain along with Torula yeast and dehydrated leaf meal. Mixture 9′ was prepared with whole ground maize and sorghum to facilitate animal feeding experiments. Mixture 9A used maize and sorghum, cooked without the addition of lime, substituted synthetic vitamin A acetate for dehydrated leaf meal, and had 1 % calcium carbonate added as a source of calcium.

The formula at present employed for commercial distribution is 9B, which uses uncooked maize and sorghum. It consists of 29% ground maize, 29% ground sorghum, 38% cottonseed flour, 3% Torula yeast, 1 % calcium carbonate and 4500 IU of vitamin A per 100 g. Type CF-2 Torula supplied by the Lakes States Yeast Corporation or its equivalent is specified. All ingredients must pass an 80-mesh screen. For all practical purposes, the protein quality of the variations in table 1 is identical. Formula 9B avoids the expense of cooking the maize and sorghum, eliminates the unstable and relatively costly leaf meal as an ingredient, and still supplies suitable amounts of calcium and vitamin A. It represents the minimum cost which could be achieved with the available raw materials.

The proximate composition and vitamin and mineral content of INCAP Vegetable Mixture 9B are given in table 2, and the amino acid composition in table 3. By comparison with the FAO Reference Protein, it has a tryptophan score of 61, a methionine-cystine score of 77, and lysine should not be limiting. These figures are markedly lower than those indicated by the results of

TABLE 2. *Composition of INCAP Vegetable Mixture 9B*

Nutrient	Per 100 g	Nutrient	Per 100 g
Moisture	7.6 g	Thiamine	2.1 mg
Protein	27.5 g	Riboflavin	1.1 mg
Ether extract	4.2 g	Nicotinic acid	7.8 mg
Ash	3.5 g	Vitamin A acetate	4500 IU
Crude fiber	2.4 g	Calcium	0.5 g
Carbohydrate	54.8 g	Phosphorus	0.8 g
Calories	370	Iron	6.2 mg

TABLE 3. *Essential Amino Acid Composition of INCAP Vegetable Mixture 9B*

Amino Acid	g/100 g	% FAO Pattern	Amino Acid	g/100 g	% FAO Pattern
Arginine	2.34		Phenylalanine	1.52	192
Histidine	1.00		Total sulphur A.A	0.92	77
Isoleucine	1.12	94	Threonine	0.87	110
Leucine	2.08	154	Tryptophan	0.24	61
Lysine	1.53	129	Valine	1.14	96

TABLE 4. *Representative Rat Growth Experiment with INCAP Vegetable Mixture 9′ (21 Days—5 Rats/Group)*

Protein in Diet, %	Average Weight Gain, g		Protein Efficiency*	
	V.M. 9′	Casein	V.M.9′	Casein
5	22	22	1.88	2.14
10	66	67	2.30	2.38
15	104	105	2.11	2.31
20	114	117	1.75	2.00
25	115	121	1.47	1.65

* Av. Wt. Gain/Av. Protein Consumed.

TABLE 5. *Representative Rat Repletion Study: Amino Acid Supplementation of INCAP Vegetable Mixture 9′ (10% Protein in Diet, 6 Rats/Group)*

A.A. Added	g % of Diet	Repletion Gain in 14 Days, g
None	0	51
L-Lysine HCl	0.1	60
L-Lysine HCl	0.2	71
L-Lysine HCl	0.3	62
DL-Methionine	0.1	53
DL-Methionine	0.2	51
DL-Methionine	0.3	53
L-Lysine HCl	0.1	64
+ DL-Methionine	0.1	
L-Lysine HCl	0.2	69
+ DL-Methionine	0.2	
L-Lysine HCl	0.3	67
+ DL-Methionine	0.3	

subsequent biological and clinical trials. Up to the present time, 10 biological trials have been completed in rats, using some 600 animals; 20 have involved some 1000 baby chicks, and 96 nitrogen balance periods in 9 children have been studied.

These biological trials will be reported in detail elsewhere, but the results of a representative rat growth experiment are shown in table 4. The average weight gains of rats fed diets varying from 5 to 25% protein, are essentially the same whether the protein is provided by Mixture 9 or casein. Protein efficiencies, however, tend to be slightly higher when casein is the protein source. When Formula 9′ is varied by using either 56% maize or 56% sorghum as compared with an equal mixture of 28% of each, identical results are obtained. Moreover, anyone of these 3 variations gives the same result at the 10% level of protein in the diet as when skim milk is the protein source.

Table 5 gives the results of a representative rat repletion study in which the effect of adding lysine and methionine to INCAP Vegetable Mixture 9 is measured in rats fed at the 10% protein level. There is no effect of methionine, but 0.2% L-lysine HCl produces a significant improvement in growth which is not increased by doubling or tripling the amount of lysine added. There is no further increase in growth when lysine and methionine are added together, compared with the effect of lysine alone. At higher levels of protein in the diet, this effect of added lysine disappears.

Representative data obtained from chick growth trials are shown in table 6. As with rats, it is possible to use either 56% maize or 56% sorghum instead of the equal mixture of maize and sorghum, without any detectable difference in growth. With chicks, however, the results with these variations of Mixture 9 are not as good as those obtained with commercial poultry food. The second half of table 6 indicates that this is due to a deficiency of lysine and methionine for optimum chick growth. The addition of 0.3% of DL-methionine and 0.2% L-lysine HCl together gives much better growth than the unsupplemented mixture. Similarly, the feed efficiency, expressed as grams of feed consumed per gram of weight gained, is improved by the amino acid supplementation, to the point where it is the same as obtained with a good commercial poultry ration.

TABLE 6. *Representative Chick Growth Trials with INCAP Vegetable Mixture 9′ (35 Days—20 Chicks/Group)*

Diet	Protein in Diet, %	Final Weight, g	Feed Efficiency
V.M. 9′	23.8	460*	2.25
V.M. 9′ with 56% maize	23.5	479*	2.31
V.M. 9′ with 56% sorghum	24.1	479*	2.27
'Ace-Hi'	23.9	587*	2.01
V.M. 9′	23.0	310†	2.45
V.M. 9′ + 0.3% DL-met	23.0	361†	2.26
V.M. 9′ + 0.2% L-lys HCl	23.0	472†	2.14
V.M. 9′ + 0.3 L-lys HCl	23.0	494†	2.07
V.M. 9′ + 0.3 DL-met + 0.2 L-lys HCl	23.0	490†	2.04

* 55 g initial weight. † 45 g initial weight.

TABLE 7. *Comparison of Nitrogen Balance in Preschool Children*

	Milk	INCAP VM-9
No. of children	9	9
Balance periods	48	48
Average protein intake g/kg/day	2.3	2.3
Average % absorbed	82.6	68.9
Average % retained*	16.3	17.8

* Difference in retention is not significant.

Nitrogen balance studies carried out in children are summarized in table 7. At an average protein intake of 2.3 g/kg, there is no significant difference in nitrogen retention whether the protein is furnished by cow's milk or by Mixture 9, even though the percentage of nitrogen absorbed is consistently less with the latter. Five children with acute kwashiorkor have been treated with Mixture 9 from the time of admission, with results fully equivalent to those previously obtained with Mixture 8 and similar to those with milk in all respects except for the slightly slower rate of serum protein regeneration previously mentioned. One such child before and after treatment with Mixture 9 is illustrated in figure 1.

The next step was the determination of its acceptability under field conditions. Seventy-six needy families in 4 Guatemalan communities were selected by local health centers and sufficient Mixture 9 provided for each preschool child to drink 3 glasses daily for 17–19 weeks. Initial acceptance was extremely good and tended to improve further during the trial period. Ninety-nine children out of a total of 129 consumed an average of 2 or more glasses daily throughout the entire period; during the final 2 weeks, 110 consumed 2 or more glasses daily.

On the basis of these encouraging results, an experimental sales trial was begun in the village of Palin with a predominantly Indian population of about 4000. The mixture was marketed under the name of Incaparina at 3 cents for a 75 g bag, sufficient for 3 glasses of a drink with the same protein quantity and quality as an equivalent amount of milk. During the 5-month trial period, sales early stabilized at 1200 bags a week; the product was recommended by the health center and school teachers but not was commercially advertised. The name Incaparina has been retained and the product is on commercial sale in Guatemala with wide and enthusiastic acceptance. Arrangements have been made for its manufacture and sale in the INCAP member countries of El Salvador, Honduras and Nicaragua and any country with the need and necessary raw materials will be encouraged to make use of the formula. A group has already begun to produce and market it for needy individuals and institutions in the southern United States.

In Central America, Incaparina is most commonly consumed as an 'atole,' a thin gruel made by adding 1

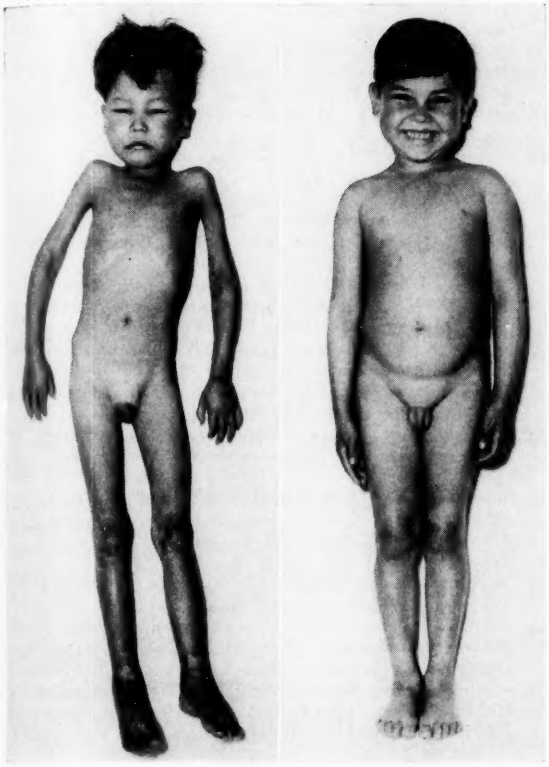

FIG. 1. Child with kwashiorkor treated with INCAP vegetable mixture 9.

glass of water for each 25 g of mixture and cooking it for 15 minutes. It is flavored to taste with sugar and cinnamon, vanilla, anise or chocolate and served either hot or cold. Incaparina can also be substituted for ⅔ of the flour in most nonbread recipes calling for wheat flour and can be made into puddings as well as used for enriching soups. It has a high acceptability for mass feeding and is a means of stretching limited institutional food budgets without lowering the nutritional value of the diet. Whether or not it is found to be useful outside the INCAP area, the results to date prove conclusively that a highly acceptable low-cost vegetable mixture of high nutritive value can be formulated and can find a useful place in national efforts to prevent protein malnutrition. It is certain that products similar to Incaparina can be developed in other areas by taking advantage of those local food sources most readily available at low cost.

SUMMARY

From a practical point of view the elaboration of vegetable protein mixture for human feeding involves the combination of cereal grains with other foods which increase the quantity and improve the quality of protein in the diet. Among the possible vegetable foods which could be used are legume seeds, oil seeds, nuts, palm kernels and leaf proteins. The concentrated protein sources which have received the most attention in this regard are the common bean, cow pea, Bengal gram (chick pea), soybean flour, peanut flour, cottonseed flour and copra proteins.

INCAP Vegetable Mixture 8 made up of 50% lime-treated maize (masa) flour, 35% sesame flour, 9% cottonseed flour, 3% Torula yeast and 3% Kikuyu leaf meal, has a protein content of 25.1% which in growth experiments in rats and chicks, and metabolic balance trials in children recovering from protein malnutrition, closely approximates the quality of milk protein. INCAP Vegetable Mixture 9B, containing 29% whole ground maize, 29% whole ground sorghum grain, 38% cottonseed flour, 3% Torula yeast, 1% calcium carbonate and 4500 units of added vitamin A/100 g, has a protein content of 27.5% and is similar in protein quality to Mixture 8 and milk. It can be produced at very low cost, and in the form of a thin gruel (atole), is highly acceptable in Central America. Its favorable initial commercial acceptance under the name 'Incaparina' suggests that it will make a significant contribution to the problem of preventing protein malnutrition in this area. It is hoped that it will also prove useful for other technically underdeveloped areas with similar economic, agricultural and health problems.

The development and testing of INCAP Vegetable Mixture 9 has at one time or another involved most of the INCAP professional staff. Dr. Edgar Braham, Mr. Alvaro Aguirre and Mr. Luiz Elías were particularly helpful in the biological trials in rats and chicks. The therapeutic and metabolic studies in children were begun by Dr. Moisés Béhar and continued by Dr. Dorothy Wilson. Dr. Miguel Guzmán planned the field and stability trials and Dr. Romeo de León supervised the field trials. Miss Susana Icaza has been responsible for cooking tests and the development of recipes and nutrition education material. Miss Raquel Flores assisted in preparation of the references for this manuscript.

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Valeur protéique de l'alimentation dans les pays tropicaux et subtropicaux

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LES TRAVAUX RÉCENTS sur le besoin en protéines alimentaires et sur leur valeur nutritive, permettent de confronter la valeur protéique de l'alimentation, dans certains pays, aux besoins protéiques.

Le présent travail a pour but de voir si quelques enseignements pratiques pour l'orientation de la production agricole peuvent se déduire de cette confrontation.

I. MÉTHODES D'ÉVALUATION

On dispose à l'heure actuelle d'un grand nombre de méthodes pour étudier l'aptitude des aliments à couvrir le besoin qualitatif d'azote. Les unes utilisent l'animal, et certaines d'entre elles sont applicables à l'homme:

—méthode des poids (coefficient d'efficacité protidique)
—bilans (digestibilité et valeur nutritive)
—régénération après déplétion
—régénération des protides hépatiques ou sanguines
—activité des systèmes enzymatiques
—analyse de la carcasse ("net protein utilization" de Miller et Bender (1, 8)

D'autres sont basées sur le dosage des amino-acides par rapport à un étalon de référence "chemical score" de Mitchell et Block (9) "Index d'Amino-Acides Essentiels (A.A.E.I.) de Oser (13).

Enfin, des travaux en cours visent à mettre au point des méthodes microbiologiques de mesure de la digestibilité azotée et de la valeur biologique des protéines (Adrian et Rerat).

Chacune de ces méthodes a ses avantages et ses défauts. Aux méthodes chimiques deux critiques ont été faites:

—le choix de la protéine de référence;
—l'hydrolyse chimique des protéines, qui, fondamentalement différente de l'action des sucs digestifs, ne rend pas compte de la disponibilité des acides aminés.

Parmi les méthodes biologiques, les bilans sont plus significatifs que les variations de poids, mais la difficulté d'une récolte strictement quantitative des excreta met souvent la technique en défaut. Les procédés de régénération après déplétion s'effectuent sur des animaux traumatisés dont le comportement peut être faussé. De l'ensemble des méthodes biologiques, celle de Miller et Bender paraît actuellement une des meilleures, en raison de sa spécificité et de sa simplicité.

Dans l'ensemble, le même classement des protides alimentaires est obtenu, quelle que soit la méthode utilisée. Certes, la place qu'occupe un protide donné dans le classement général n'est pas nécessairement la même selon que l'on utilise la méthode des poids, la technique des bilans ou la méthode chimique. Mais ce ne sont là que des exceptions et le sens général de la hiérarchie des protéines est en gros valable pour tous les mono-gastriques. Il souligne la supériorité incontestable des protéines d'origine animale.

La connaissance de la valeur biologique et la digestibilité permet d'affecter à chaque aliment protéique un "coefficient d'utilisation protéique." Celui-ci permet de

TABLEAU 1

Aliments	Prot. Bruts g/100	CUP*	VPN†	Cal. des Prot.	Cal. p/100 g	$\frac{\text{Cal. Prot.}}{\text{Cal. Tot.}} \times 100$
Farine de manioc	1,5	50	0,75	3,0	338	0,9
Patate douce	1,3	41	0,53	2,1	117	1,8
Riz blanc	6,7	70	4,7	18,8	360	5,2
Maïs	9,5	49	4,7	18,8	356	5,3
Millet	9,7	51	4,5	18,0	340	5,3
Farine de blé	10,9	52	5,7	22,8	370	6,2
Sésame	18	57	10	40	574	7,0
Haricot	22	32	7	28	345	8,1
Farine de pois	26	39	10	40	363	11
Arachide (farine délip.)	53	50	26	104	325	32
Poudre de lait écremé	36	80	29	116	360	32
Sésame (farine délip.)	40	60	28	112	340	33
Tournesol (farine délip.)	50	61	30	120	300	40
Coton (farine délip.)	57	57	32	128	295	43
Soja (farine délip.)	46	72	33	132	261	50
Poisson (farine délip.)	75	70	52	208	300	69

* CUP—Coefficient d'utilisation protéique. † VPN—Valeur protéique nette.

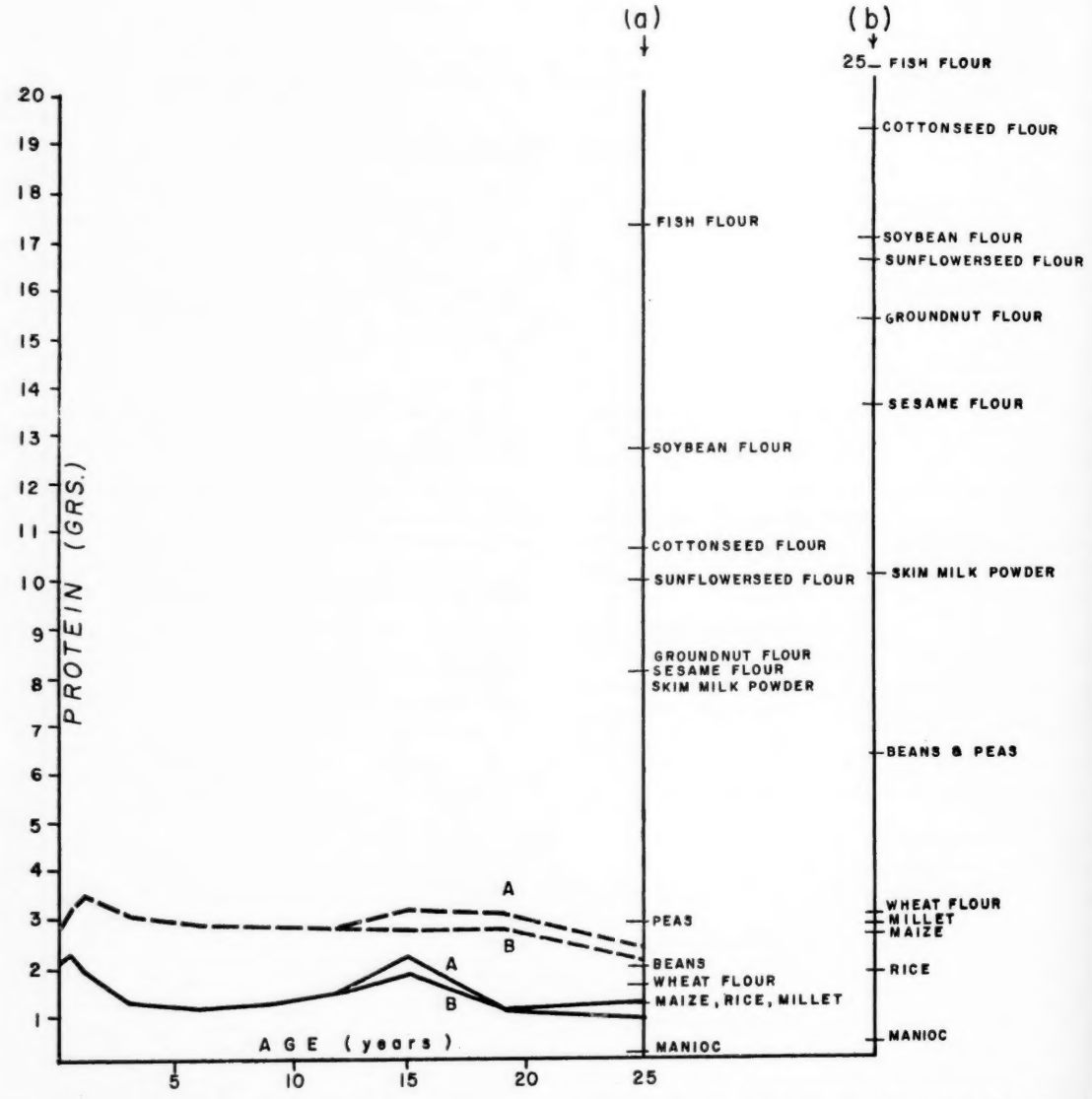

FIG. 1. *Protein requirements per 100 calories according to age.* —— Requirements in terms of FAO reference protein (including allowance for individual variations); ----- NRC allowances; *A:* females; *B:* males. On the right hand side of the figure is indicated the yield of protein per 100 calories of some foodstuffs in terms of (*a*) net protein value, (*b*) crude protein.

calculer, compte tenu de la teneur brute de l'aliment en protéines, la valeur protéique nette de l'aliment.

Le tableau 1, établi à l'aide des Tables de Composition des Aliments de la FAO (6) et des Coefficients d'Utilisation Protéique cités par la littérature—notamment par Block et Mitchell (2), Platt et Miller (16)—donne les valeurs protéiques nettes de divers aliments de base utilisés dans les pays tropicaux, et de certains aliments de supplémentation dont on vise à accroître la consommation. Nous en tirerons plus tard les conclusions qui s'imposent.

La notion de 'valeur protéique nette' d'un aliment permet de juger de l'aptitude de cet aliment à couvrir les besoins en protéines selon l'âge et les conditions physiologiques. En 1952, l'un de nous avait, avec Brock, établi un graphique qui visait à cette fin mais utilisait des teneurs en protéines brutes (3). Il nous a semblé utile de recalculer ce graphique en tenant compte non plus de la teneur brute des aliments en protéines, mais de la teneur en protéines nettes utilisables (voir fig. 1).

Par la même occasion, au lieu d'utiliser les normes recommandées par NRC, comme nous l'avions fait, nous utilisons aussi les normes recommandées par la FAO; la valeur protéique nette d'une protéine permettant de passer du besoin en une protéine de référence aux besoins en une protéine quelconque (17, 18).

II. ESTIMATION ET COUVERTURE DU BESOIN AZOTÉ DE L'HOMME

Etant données les valeurs biologiques différentes des protides alimentaires, le besoin azoté ne peut s'exprimer par un chiffre unique. Généralement, les extrêmes pour l'homme de 70 kg se situent par calorie basale à 4,76 mg d'azote sous forme de protéines de farine blanche, et à 2,76 mg sous forme de protéines du lait. Historiquement parlant, deux courants opposés s'affrontent depuis le début du siècle: Ceux qui fixent le besoin d'après les taux réellement consommés par des individus actifs en bonne santé, et ceux qui le chiffrent d'après les quantités minima susceptibles d'equilibrer le bilan. Les premiers arrivaient à des valeurs variant de 70 à 100 g de protéines par jour pour l'homme adulte; les seconds concluaient à des quantités nettement plus faibles. Les experts de la FAO ont tenté de concilier les deux points de vue en définissant le besoin pour une protéine de référence et en employant des coefficients d'ajustement tenant compte du 'protein score.'

Il s'agit en fait de couvrir, à l'aide de la protéine de référence, les différents besoins spécifiques en chacun des acides aminés indispensables pour l'homme. Les estimations que l'on trouve dans la littérature ne sont pas toujours concordantes et de plus il faut souligner que les conditions dans lesquelles furent déterminés ces besoins ne sont pas celles de l'alimentation normale qui met en jeu des phénomènes d'antagonisme (déséquilibre par excès, indisponibilité par réaction de Maillard) ou de synergie (effet d'épargne des calories et des acides aminés non indispensables). On doit donc raisonner avec beaucoup de prudence et se servir de la notion de besoin minimum d'acides aminés comme d'un fil conducteur mais non comme d'une norme impérative. C'est pourquoi les besoins moyens en protéine de référence s'accompagnent d'allocations pratiques de sécurité.

Avec toutes les réserves qui s'imposent, la FAO a établi une courbe provisoire des besoins en protéine de référence en fonction de l'âge, et définit une allocation de sécurité qu'on peut résumer ainsi pour les exigences de ce qui va suivre:

Protéine de Référence

	Besoin Minimum	Allocations de Sécurité
9 mois	1,4	2,1
2 ans	1,1	1,65
5 ans	0,8	1,2
9 ans	0,7	1,05
Adolescents (14 ans)	0,8	1,2
Adultes	0,35	0,52

III. APTITUDE D'UN ALIMENT À COUVRIR LE BESOIN PROTÉIQUE

Ces besoins peuvent, comme nous l'avions fait en 1950, se calculer, soit en grammes de protéines par 100 calories apportées (3), soit, comme l'ont indiqué Platt et Miller (15), en calories provenant des protéines nettes utilisables par 100 calories totales. On peut aussi exprimer les besoins non plus en gramme de protéine par kilo de poids actuel, mais en grammes par jour. Pour ce faire, nous avons utilisé les poids de référence donnés par la FAO (4), ou, à défaut, ceux donnés par NRC (18), et les Besoins en Calories de la FAO (4) ajustés à la température de 25°.

Pour que les besoins caloriques et protéiques (besoin minimum et allocations protéiques de sécurité) soient couverts en même temps aux différents âges, il faut que les aliments présentent un rapport $\frac{\text{calories protéiques}}{\text{calories totales}} \geqslant$ aux chiffres donnés dans la colonne de droite du tableau 2.

La confrontation des tableaux 1 (partie supérieure) et 2, et la lecture des graphiques 1 et 2 conduisent aux mêmes conclusions, à savoir:

1) les racines féculentes (manioc, patates douces, ignames, taros) présentent une grave insuffisance du rapport $\frac{\text{calories protéiques}}{\text{calories totales}}$.

2) les céréales ont un rapport à peine tangent au besoin minimum de l'enfant de 2 ans ou de 14 ans. Elles sont inaptes à couvrir l'allocation de sécurité, sauf chez l'adulte normal;

3) seuls les aliments de la partie inférieure du tableau 1 ont un rapport satisfaisant en tous temps, sauf haricots et sésame pour l'allocation de sécurité du très jeune enfant et de la nourrice;

4) tous les aliments de la partie inférieure du tableau 1, à la dose où ils couvrent le besoin en protéines, sont incapables de couvrir le besoin énergétique.

TABLEAU 2

Âge	Poids, kg	Besoins Prot. Réf. par kg	Besoins Prot. Réf. par Jour	Calories des Prot. x4	Besoins en Cal. à 25°	$\frac{\text{Cal. Prot.}}{\text{Cal. Tot.}} \times 100$
A/— Besoin minimum						
9 mois	9	1,4	12,6	50,4	1120	4,5
2 ans	12	1,1	13,2	52,8	1203	4,4
5 ans	18	0,8	14,4	57,6	1573	3,7
9 ans	27	0,7	18,9	75,6	1943	3,9
14 ans	49	0,8	39,2	156,8	2868	5,5
adulte h. 25 ans	65	0,35	22,7	90,8	2960	3,1
femme all. 25 ans	55	1,4	77,0	308,0	3127	9,8
B/—Allocations de securite						
9 mois	9	2,1	18,9	75,6	1120	6,7
2 ans	12	1,65	19,8	79,2	1203	6,6
5 ans	18	1,2	21,6	86,4	1573	5,5
9 ans	27	1,05	28,3	113,2	1943	5,8
14 ans	49	1,2	58,8	235,2	2868	8,2
adulte h. 25 ans	65	0,52	33,8	135,2	2960	4,6
femme all. 25 ans	55	2,1	115,5	462,0	3127	14,8

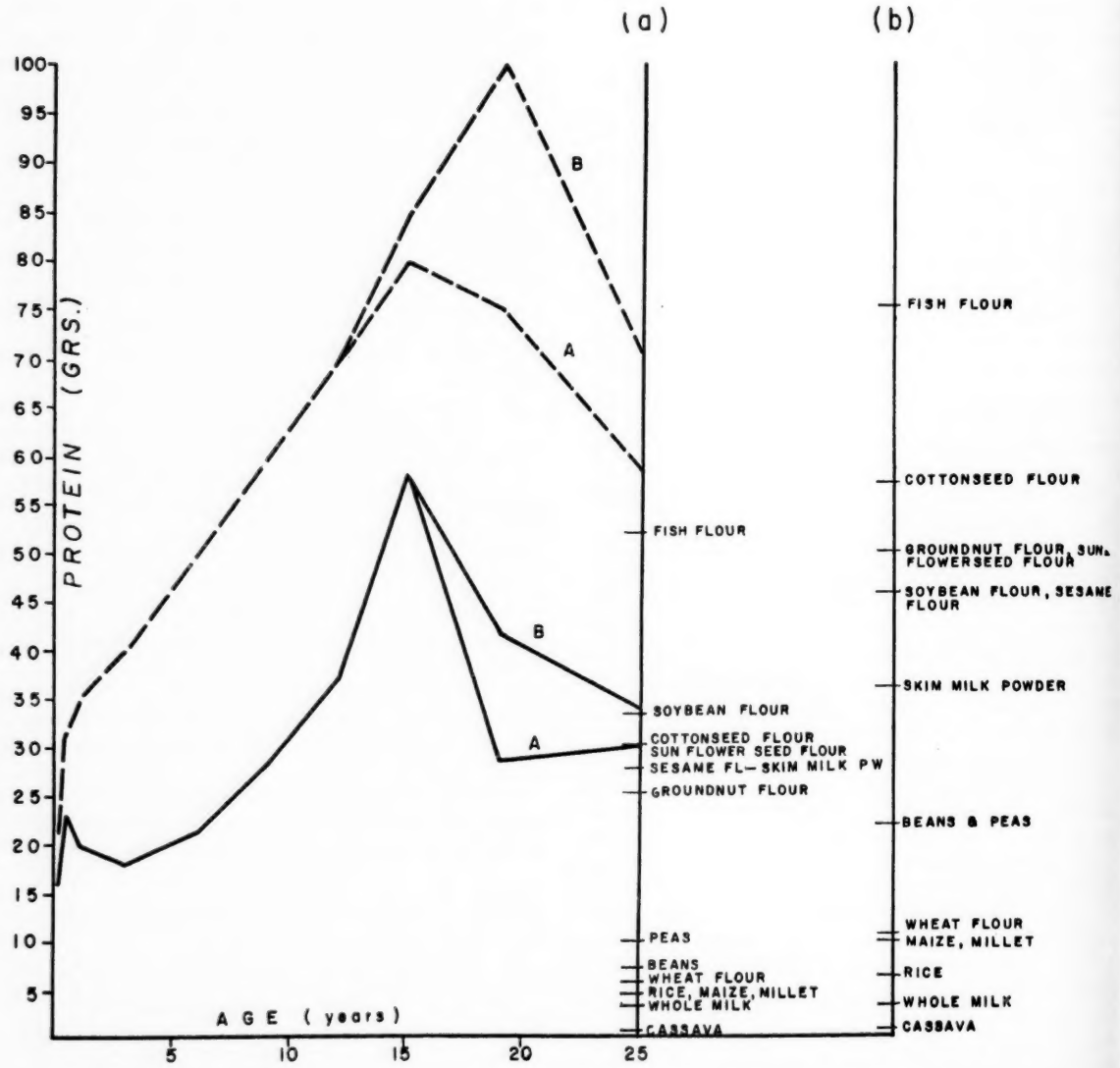

FIG. 2. *Daily protein requirements, according to age.* —— Requirements in terms of FAO reference protein (including allowance for individual variations); ----- NRC allowances; *A:* females; *B:* males. On the right hand side of the figure are indicated (*a*) net protein value, and (*b*) crude protein, per 100 g of some foodstuffs.

5) Cette façon de présenter les faits ne tient pas compte de la supplémentation.

IV. ÉTUDE DE LA VALEUR PROTÉIQUE DE L'ALIMENTATION

Pour éviter ces inconvénients Platt et Miller (16) recommandent la détermination de la valeur protéique nette du régime alimentaire, c'est-à-dire d'étudier globalement la ration moyenne quotidienne. C'est là évidemment *la methode la plus rigoureuse* en ce sens qu'elle tient vraiment compte de la composition réelle de l'alimentation, des effets du traitement sur les protéines et sur la disponibilité des acides aminés; elle indique le coefficient d'utilisation protéique de la ration et, par conséquent, connaissant le taux protéique de la ration, on peut calculer la valeur protéique nette de l'alimentation et calculer le coefficient à appliquer à l'ensemble de la ration pour couvrir, s'il y a lieu, le besoin protéique.

Une autre méthode est celle recommandée par la FAO qui consiste à calculer la teneur en acides aminés des aliments composant la ration et à la comparer aux acides aminés d'une protéine de référence entièrement fabriquée hypothètiquement (on pourrait tout aussi bien la comparer aux acides aminés des protéines du lait ou de l'oeuf, prises comme éléments de référence). Cette méthode exige la connaissance de la composition en acides aminés de tous les aliments consommés; en outre, elle ne tient pas compte des effets éventuels du traitement pour ces acides aminés, mais elle permet de calculer d'une manière pratique la façon la plus économique de

couvrir le besoin protéique par le jeu de la supplémentation.

Nous avons appliqué cette, technique à un certain nombre de rations des pays tropicaux et sub-tropicaux. Les résultats, établis pour des régimes décrits dans le tableau 3 sont consignés dans le tableau 4.

Ces résultats appellent quelques commentaires.

Les "scores" obtenus sont des *maxima*. En effet, le calcul ne peut faire intervenir la non-disponibilité des acides aminés soit naturelle soit due au traitement des aliments. Le régime alimentaire a été calculé sur des moyennes d'enquête de 3 à 7 jours; on fait ainsi intervenir chaque jour des aliments qui ne sont consommés qu'une ou deux fois durant l'enquête, réalisant ainsi une supplémentation artificielle qui, en fait, n'existe pas.

La technique suivie ne tient pas compte non plus du facteur 'temps.'

Ces résultats sont donc supérieurs aux valeurs que l'on aurait obtenues en déterminant par la méthode de Bender et Miller la valeur protéique réelle d'une ration/jour dans un pays tropical. En effet, pour des régimes de Nigeria, Platt et Miller obtiennent pour l'utilisation protéique nette, des valeurs allant de 40 à 59 (16). Hugues confirme que, pour les régimes végétariens ou pauvres en protéines, la différence est sensible entre les taux d'acides aminés calculés et ceux obtenus par l'analyse (7).

Ces réserves faites, voyons néanmoins les déficits de ces régimes. Il ressort de l'étude des tableaux 3 et 4 que:

1) les facteurs limitants primaires sont toujours les acides aminés soufrés, et particulièrement la méthionine;

2) le facteur limitant secondaire est généralement le tryptophane;

3) contrairement à ce que l'on écrit généralement, la lysine n'est pas un facteur limitant important. même dans les régimes à base de céréales. Cela ne tient pas à un taux sous-estimé de la lysine dans la protéine hypothétique de référence; en effet, le résultat est le même si on le compare aux protéines de l'oeuf;

4) certains aliments d'appoint, consommés en petites quantités et généralement considérés comme très secondaires pour les ménagères, interviennent d'une manière non négligeable dans la valorisation de la ration protéique. S'il est connu par exemple que les viandes, les oeufs, les produits laitiers, diminuent le déficit en acides aminés soufrés, il en est d'autres, d'origine végétale qui sont au moins aussi bien pourvus en méthionine et cystine (12) comme le montre le tableau 5. Parmi ceux-ci, une céréale, le "fonio" qui "résiste très bien à la sécheresse et vient dans des terres superficielles de mauvaise qualité" est remarquablement bien pourvue (5, 19).

TABLEAU 3. *Consommation Alimentaire*

	1 Sahel Adultes m.	2 Savane pers/ Jour	3 Savane Arb. Adultes m.	4 Forêt pers/ Jour	5 Forêt pers/ Jour	6 R. Côtière pers/ Jour
Sorgho et mils	712	269	81		28	
Riz		12		94		
Maïs			24	49	20	210
Bière de mil		303				
Igname		20	1417	50		
Manioc	11		20	177	102	29
Semoule de manioc		1		13		308
Patate douce		28			110	
Coleus		4				
Taro				353		
Banane plantain				73	1831	
Arachide	11	18	3		6	0,8
Voandzou		73				
Haricot	13	34	4	6	15	6
Divers		7	1	18		
Karité		3				
Palme				44		29
Coco				2		7
Piment	3	3	3	6		13
Gombo	25	25	19	4		10
Tomate			3	10		18
Aubergines	1			6		6
Feuilles	2	8	19	1		31
Oignon	2,5					
Divers		2				
Fruits		27	18	10		16
Kola	6	2	11			
Dattes	3					
Poisson frais	58	0,1			17	
Poisson, fumé, séché				10		23
Crustacés						1
Viande d'élevage	15	5	4	3	25	5
Viande de chasse		0,5		12	1	
Volaille	3,5	5		4		
Lait	95		2	0,7	91	
Protéines	112,3	54,6	52,0	39,7	41,6	40,8
Besoin protéique NRC		65,6		59,4		59,4
Calories	2980	1702	2430	1891	2462	2067
Besoin calorique	2561	2106	2060	1965		1993

V. CONCLUSIONS PRATIQUES

Elles sont simples: Si l'on voulait couvrir le besoin protéique en maintenant le type d'alimentation actuel, on arriverait à des taux caloriques excessifs et à un gaspillage de certains acides aminés. Dans le cas des régimes nigériens, déterminés directement par Platt et Miller (16), on arriverait à multiplier les rations actuelles par 1,66 à 2,5. Dans le cas de nos rations calculées, le coefficient croît de 1,25 à 2.

La supplémentation physiologique du nutritionniste signifie pour l'agronome le développement sélectif de la production. Il est en effet impossible de penser à doubler une production totale. Il faut donc se décider au plus tôt à accroître la production d'aliments de supplémentation.

Dans les pays de la zone tropicale humide où racines,

TABLEAU 4

	1 Sahel Céréales Prot. Animaux		2 Savane Céréales Légumineuses		3 Savane Arb. Féculents Céréales		4 Forêt Féculents Céréales		5 Forêt Féculents Céréales		6 Région côtière Féculents Céréales	
	consom.	score	consom.	score	consom.	score	consom.	score	consom.	score	consom.	score
Isoleucine	5,94	122	3,58	156	2,52	111	1,85	111	2,24	128	1,78	104
Leucine	14,58	264	4,83	184	5,05	196	3,07	161	3,24	162	3,86	198
Lysine	5,04	104	3,12	136	2,38	105	2,18	131	2,29	131	2,09	122
Phénylalanine	5,24	162	3,31	216	2,33	154	1,83	164	2,00	172	1,60	140
Tyrosine			1,50	98			0,96	86	0,88	75	1,67	146
Méthionine + cystine	3,92	80	1,80	(78)	1,14	(50)	1,08	(65)	1,19	68	1,27	74
Méthionine	2,21	85	0,95	79	0,78	65	0,69	79	0,57	(62)	0,78	86
Thréonine	4,25	131	2,31	150	1,76	116	1,50	135	1,47	126	1,57	138
Tryptophane	1,24	(77)	0,73	96	0,77	102	0,44	80	0,46	79	0,39	(68)
Valine	6,31	129	3,64	158	2,46	109	2,03	122	2,56	147	1,94	113
Protéines g	112,3		54,6		52,0		39,7		41,6		40,8	
PTU* en g par jour	86,4		42,6		26,00		25,8		25,8		27,7	

( ) Facteur limitant primaire, — Facteur limitant secondaire. * Protéine theoriquement utilisable: 1, Jarawaji (Nigeria) (10); 2, Bombouaka (Togo) (14); 3, Bero Okuta (Nigeria) (10); 4, Kouma Adamé (Togo) (14); 5, Masaka (Ouganda) (11); 6, Attitogon (Togo) (14).

TABLEAU 5. *Comparaison de Quelques Aliments Riches en Acides Aminés Soufrés (Méthionine + Cystine)*

	Teneur en g par g d'Azote Total	Teneur en g pour 100 g de Partie Comestible
Dolichos biflorus (grain de cheval)	0,224	0,774
Anacardium occidentale (pomme cajou)	0,252	0,880
Sesamum indicum (sésame)	0,311	1,132
Soja	0,195	1,191
Bertholletia excelsa (noix du Brésil)	0,548	1,445
Digitaria exilis (Fonio)	0,525	0,663
Poisson	0,266	0,873
Poulet	0,247	0,814
Oeuf	0,342	0,700

Des aliments de ce type permettent de réaliser une supplementation du régime alimentaire qui conduit, comme le montre le tableau 6, à une amélioration sensible de la PTU.

TABLEAU 6. *Exemples de Supplémentation des Rations 2 et 4*

	Ration (2)	Ration (2) + 70 g Sésame	Ration (4)	Ration (4) + 25 g Farine de Poisson	Ration (4) + 50 g Sésame + 15 g Poisson − 177 Manioc
	score	score	score	score	score
Isoleucine	156	148	111	114	115
Leucine	184	184	161	161	166
Lysine	136	123	131	156	137
Phénylalanine	216	228	164	160	181
Tyrosine	98	114	86		
Méthionine + cystine	(78)	(91)	(65)	(78)	(86)
Méthionine	79	93	79	98	104
Thréonine	150	148	135	143	140
Tryptophane	96	101	80	83	89
Valine	158	149	122	128	125
Calories	1702	2106	1891	1974	1970
Besoins FAO	2106	2106	1965	1965	1965
Protides	54.6	68.2	39.7	60.1	59.7
Besoins NRC	65.6	65.6	59.4	59.4	59.4
PTU g jour	42.6	62.0	25.8	46.9	51.3

PTU = Protéine theoriquement utilisable.

La supplémentation du régime 2 par 70 g de sésame, et celle du régime 4 par 25 g de farine de poisson, fait passer les indices protéiques de 79 (méthionine dans les 2 cas) à 93 et 98. L'autre facteur limitant (acides aminés soufrés), passe de 78 et 65 à 91 et 78.

La colonne 5 du Tableau 6 montre qu'une poly-supplémentation donnes des résultats encore meilleurs.

tubercules et bananes plantain prédominent toujours, il est nécessaire de développer d'abord de nouveaux aliments à haute teneur en protéines, particulièrement les graînes de légumineuses, pour relever le niveau azoté global. Cela exige en second lieu la production d'aliments d'appoint riches en méthionine et en tryptophane.

Dans les régions plus sèches où les céréales sont les aliments de base et où les légumineuses constituent parfois un appoint non négligeable, le niveau protéique serait satisfaisant si la valeur protéique nette de la ration était relevée par le jeu de la supplémentation. Pour l'agriculture, celà signifie qu'il faut développer et diversifier les apports dits secondaires: cultures de jardin, champs de case, petit élevage domestique, pêche et pisciculture, dérivés des graines oléagineuses, au lieu de s'attacher à accroître les aliments de base

Cette étude fait ressortir en particulier l'importance plus grande qu'on ne le croit généralement, de la mise au point d'aliments riches en protéines pour l'enfant et la famille, et justifie les efforts et les fonds investis depuis 6 ans dans ces recherches.

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Some aspects of protein malnutrition in childhood

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THE MAIN FACTORS which determine the nutritional status of the members of a community, may be classified as follows (44):

Factors which determine availability of food
- Production
- Transportation
- Storage

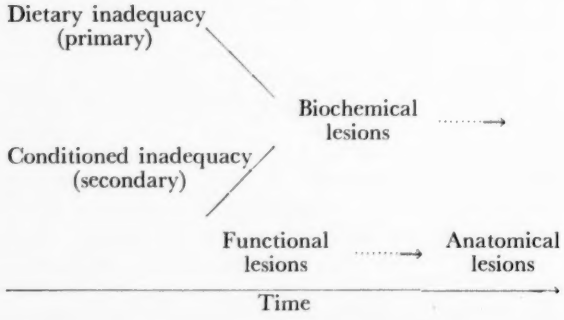
Factors which determine consumption of food
- Economical
- Cultural
- Psychological

Factors which determine utilization of food
- Physiological
- Pathological
- Previous nutritional status

In great areas of the world, the algebraic sum of the magnitudes of these factors is such as to originate a social group composed of hundreds of millions of human beings, whose anthropological characteristics are those of subjects who can not cover the minimal requirements for a healthy life (21).

It seems to be an established fact, that one of the main characteristics of their diet is its insufficient content in foods of animal origin. This means, not only an inadequate provision of proteins of high biological value, but also of all the other nutrients contained in animal protein (42). Used with this in mind, the term 'protein malnutrition' is a useful abbreviation.

For many years, our group has adopted and adapted Jollife's scheme (35) to explain the pathogenesis of malnutrition, at an individual level:



The different phases in this sequence manifest themselves through various signs, which may be grouped into 3 main categories (22, 44): I, Universal; II, Circumstantial; III, Aggregated.

Universal signs as such are always present in malnutrition, regardless of its etiology, intensity or clinical appearance. At the various levels on the above scheme, these signs may be characterized as dilution, hypofunction, and atrophy. In childhood, they are mainly determined by the decrease in the normal rate of growth and development, which eventually can be severe enough to cause arrest or even regression in certain processes.

Circumstantial signs may not necessarily be present in malnourished subjects; if they are, they are easily demonstrated and helpful for diagnosis and for identification of a given clinical picture, although not for prognosis nor for selection of the most appropriate treatment. These signs are actually manifestations, at anatomical level, of universal signs, and they are easily modified by such individual factors as age, skin and hair color, the previous nutritional status of the subject, or by environmental factors such as temperature or humidity. Skin lesions and pitting edema fall into this group.

Aggregated signs are those which, although not directly due to malnutrition, frequently accompany the disease, whose clinical features they may alter, while their own are also modified from what is usual in well-nourished subjects. These signs definitely influence early prognosis and treatment in individual cases. Such is the case of acute infections and electrolyte disturbances.

Full knowledge of the particular features which these groups of signs present in different parts of the world, will undoubtedly help to clarify some of the concepts which only a few years ago, were obscure and originated misunderstandings.

Since an inadequate rate of growth and development is an universal sign of malnutrition, their assessment can be considered as an important step for obtaining an insight of the nutritional status at individual, familial and communal level. Anthropometrical studies (40, 45) have revealed that in rural as well as urban Mexican communities, children are born with measurements which do not differ from those given for newborns in technically advanced countries. Similarly, during the first 4 months of life, growth rates can be considered as very satisfactory, and, if only weight is considered, it may exceed the patterns established for normal North

American infants; these findings agree with those obtained in some parts of Africa (61). However, starting at this point there is a progressive decrease in the rates of growth, establishing a difference with the normal which becomes maximal at an age of 26 months, in the case of weight, and of 3–5 years for height. Later on, the rates become somewhat more satisfactory, as they stabilize for the weight around 90% and for the height near 96% of the normal average. Data obtained in a rural community, including all the school children, are shown in table 1. Repeated annual studies have confirmed these results. It must be said that studies conducted in normal Mexican children have shown that the maximal deviation from average height is 6% (46).

Assessment of bone age in Central America (57) has shown a certain retardation after the age of 1 year; similar findings have been made on children already sick with severe malnutrition (36).

As far as the mental aspect of development is concerned, studies with the Gesell, Terman-Merrill and Goodenough tests (52) have shown that, as reported elsewhere (17) babies are born with scores higher than normal average, only to drop rapidly and reach subnormal levels at the age of 20 months. The deficit is about 30% at 42 months of age, and continues through school age into adolescence (47). In these children, an inverse correlation was found between weight deficit and intelligence scores, as shown in table 2. It may be surmised that intelligence is being influenced by ecological factors, some of them acting directly and others, indirectly through a decrease in development potentialities.

TABLE 1. *Weight and Height Averages in Children From Tlaltizapán, Mor.*

Age	No. of Cases	Weight, Average Value, Kg	% of Normal	No. of Cases	Height, Average Value, cm	% of Normal
At birth	65	3.07	100	66	48.3	97
1 month	144	3.87	103	133	52.2	97
2 months	48	4.90	109	46	56.3	99
3 months	30	5.68	108	31	59.4	98
4 months	32	6.31	105	29	61.3	98
5 months	21	6.67	102	20	63.3	98
6 months	12	6.81	97	12	63.0	97
7 months	11	7.06	94	9	66.5	107
1 year	26	8.34	79	25	73.5	98
2 years	34	9.36	74	32	79.6	94
3 years	24	12.18	83	23	88.1	94
4 years	31	14.20	85	15	93.1	92
5 years	29	15.44	86	30	97.4	92
6 years	20	17.43	86	21	106.7	94

TABLE 2. *Correlation Between Body Weight and Intelligence Quotient in 299 School-Age Children of Tlaltizapán, Mor.*

% of Normal Weight	No. of Cases	Av. Intellectual Quotient
51–60	8	65.25
61–70	22	69.91
71–80	102	80.50
81–90	88	81.55
91–100	56	86.94
101–110	23	89.39
	299	

TABLE 3. *Average Weight of Several Organs of Malnourished Children, Expressed as Percentage of Normal*

Age, Yr.	Brain	Heart	Kidney	Spleen	Liver, (Nonfatty)
1	85	70	70	54	60
2	80	71	64	61	71
3	81	78	65	62	76
4	86	75	67	59	
5	83	74	64		
6	92	74	68	59	

TABLE 4. *Levels of Behavior in Preschool-Age Children During Recovery From Chronic Advanced Malnutrition, as Percentage of Mean Theoretical Normal for Chronological Age*

	Days After Admission							
	0	15	30	45	60	75	90	105
Motor	39	47	47	54	57	59	62	66
Adaptive	36	51	53	65	69	66	66	71
Language	28	37	38	45	46	46	45	48
Personal-social	34	43	52	60	61	64	60	61

The significance of these data may be best understood if one takes into account the dietetic history of these children. It has been shown that in spite of an adequate milk secretion rate (30) and composition (2) throughout the first year of lactation, human milk alone is insufficient to maintain an adequate growth rate beyond an age of about 20 weeks. However, few if any complementary foods are introduced, either because the child apparently satisfies his hunger, or because poor sanitary conditions give rise to repeated gastrointestinal infections, every time a food other than breast milk is given to the baby. In this fashion starts the characteristic history of infant malnutrition, where, in spite of much higher protein requirements, in poverty or richness, the child receives less food than his parents (38). Under these conditions, the child becomes progressively more sensibilized to environmental stresses, which in the future will be extremely abundant and severe. Universal signs of malnutrition, as related to slowing down or negative acceleration of the rates of growth and development, circumstantial and aggregated signs as related to ecology, will become increasingly evident, and integrate the well known clinical pictures of chronic advanced protein malnutrition. At this stage, parameters of growth and development show exaggerated deficiency in relation to that already present, height being the least affected (92% of the normal, as an average). In the same way, different organs are affected inequally as shown in table 3 (1).

Levels of behavior development, expressed as percentage of the normal theoretical average for the age on admission to the hospital and during recovery (53)

TABLE 5. *Distribution of Different Types of Chronic Advanced Malnutrition in Relation to Age*

Clinical Type	Age								Total
	1 Year		2 Years		3 Years		4 Years		
	No.	%	No.	%	No.	%	No.	%	
Kwashiorkor	14	16	233	42	99	54	53	56	399
Marasmic kwashiorkor	4	4	98	18	39	21	25	26	166
Marasmus	71	80	227	40	45	25	17	18	360
Total	89		558		183		95		925

are shown in table 4. No complete normalization is achieved, in spite of the fact that abnormal electroencephalographic tracings have been observed to regress upon recovery (14).

Although too well known in their somatic characteristics as to deserve detailed description, mention may be made of the 2 extreme clinical pictures which malnutrition shows in childhood, that is, the 'Mehlnährschaden' or 'kwashiorkor' baby, with pitting edema and striking muco-cutaneous lesions, on one side, the atrophic or marasmic type with few of them, on the other, and the 'marasmic kwashiorkor' in between. The fundamental difference among these various appearances seems to lie in the ratio of lean over fatty body mass, which may perhaps be related to different patterns of food intake and of distribution of the same during the day. In spite of their gross deficiency in caloric intake, and of the fact that they are 40% or more underweight and show striking muscular wasting, typical kwashiorkor children have considerable amounts of fat in their liver, and subcutaneously on the cheeks, abdomen and thighs. Survivors of this stage of infantile malnutrition may show a gradual disappearance of these fat depots, drifting through the stage of marasmic kwashiorkor into typical 'marasmus.' Younger children may show only this last aspect. The frequency distribution of the clinical type of advanced malnutrition according to chronological age, is shown in table 5 (48).

It must be pointed out that the different types have the same lethality, respond equally to the same treatment, and recover following a strikingly similar pattern (49).

Further study of these types of malnourished children, taking into account the hierarchy of the physical signs involved, should clarify the semiological and pathological significance of the different clinical aspects.

The signs considered as universal, can be well defined at a biochemical level. A few of the metabolic characteristics of malnourished children will be described.

PROTEIN METABOLISM

Besides the characteristic anomalies of serum protein concentration, studies of body composition performed on children who died from chronic malnutrition have demonstrated a striking protein depletion in different tissues (64), some of them being more 'labile' in this regard than others. It has already been shown that the brain is much less affected than liver, kidney and spleen; the extracellular proteins of skin and skeleton are also much more stabile. Muscle and liver proteins are among those rapidly mobilized in severe malnutrition.

Nitrogen balance studies have revealed that absorption and retention of nitrogen from a milk diet may be equal or even better than in healthy, well nourished children of the same age (23, 34, 51). Deficient absorption and retention may be observed when the diet is composed only of certain proteins of vegetable origin (24, 33).

It has been repeatedly said that nitrogen retention, as an index of tissular nitrogen depletion, may be a good biochemical index for differentiation between marasmus and kwashiorkor type childhood of malnutrition (58); however, metabolic balance measurements have revealed that, while 16 children with kwashiorkor as an average absorbed 75.4% and retained 41% of their intake, 17 babies with marasmus absorbed 74.9% and retained 38.3% (8); similar lack of influence of the clinical type of malnutrition on nitrogen balance has been reported from Africa (34). It has been hypothesized (58) that the magnitude of nitrogen retention may be related only to depletion of labile organic protein, which may be equally affected by the acute (infectious) incident which brings the child into the hospital.

This influence of diarrhea, which was present in 68% of our clinical material, was studied evaluating the percentage of absorption of ingested nitrogen in children who passed less than 100 g, and in those who passed more than 400 g of fresh feces per day (8). Nitrogen absorption in the former was of 82% of the intake, and of 75% in the latter. Analysis of variance shows that the linearity of the relation between intake and absorption does not change significantly when more feces are excreted per day. Thus, if these results can be generalized, it would appear that diarrhea per se has less influence on nitrogen metabolism than was thought, and that rather the extended practice of reducing or withholding food during diarrhea episodes is the cause of a more or less sudden lack of available precursors for protein synthesis, in an organism which already has been suffering chronic protein depletion. The unfavorable effect on growth and maintenance which intermittent feeding of protein produces in rats, may be interesting in this regard (18).

Studies with isotope dilution techniques in 14 children with chronic advanced malnutrition have been performed (19). The average half-life of albumin in these children is 9.3 days (range 6.6–11.5 days), and during recovery, 9.8 days (range 7.0–11.3 days). Besides, no difference in the vascular-extravascular distribution of albumin in these stages can be ascertained, nor are there differences in regard to clinical appearance of type of diet. Thus, unlike some other animal species, human babies seem unable to reduce their rate of protein

catabolism as nitrogen intake decreases, thus inducing necessarily an imbalance between synthesis limited by the supply of precursors, and a rate of catabolism equally suitable for a subject with an adequate diet.

Studies on blood amino acid concentration and partition (68) have shown that, regardless of the clinical type, there is marked hypoaminoacidemia, total free amino acids being lowered to about ⅓ of the normal. The lowest levels on admission correspond to tyrosine, cystine, and valine; this pattern is quickly reversed when treatment is started, and after 9 days, only phenylalanine, cystine and tyrosine remain at abnormally low levels, tyrosine being the last one to become normal. Data on urine (7, 54) have revealed, aside of increased aminoaciduria, an absolute and relatively higher excretion of isoleucine than of leucine and of phenylalanine greater than of tyrosine. Thus, the possibility of an impairment of the activity of phenylalanine hydroxylase in these malnourished children, similar to what has been found in newborn animals and in 1 human premature infant (37) may be hypothetically advanced, and if confirmed, would implicate a biochemical index for anomalous growth and development, a universal sign of malnutrition in childhood.

TABLE 6. *Relation of Intracellular Organic and Inorganic Components in Chronic Advanced Malnutrition (Average Values, mM/mM)*

Biopsy No.	Metabolite	Na	K	Mg	P'_i	P_o	P_t	Pyr	α Keto	Lact.	Cit.
					Survivors						
1	Pyr.	255	269	50	98	58	156		0.7	21	24
2	Pyr.	234	390	70	108	164	276		1.1	29	25
1	α keto	333	351	64	128	75	203	1.3		28	32
2	α keto	221	369	72	102	149	252	0.9		26	24
1	Lac.	12	12	2.3	4.6	2.7	7.3	0.05	0.03		1.1
2	Lac.	8	14	2.7	3.9	5.7	9.6	0.03	0.04		0.9
1	Cit.	10	11	2.0	4.0	2.4	6.3	0.04	0.03	0.9	
2	Cit.	9	16	3.0	4.4	6.3	10.7	0.04	0.04	1.1	
					Deaths						
1	Pyr.	169	231	40	74	127	202		1.4	23	8.7
2	Pyr.	294	544	95	246	86	352		0.6	126	30
1	α keto	117	160	28	52	88	140	0.7		6	5.8
2	α keto	178	900	158	407	42	545	1.6		208	44
1	Lac.	7	10	1.7	3.2	5.5	8.7	0.04	0.06		0.4
2	Lac.	8	4	0.7	2.0	0.7	2.7	0.01	0.008		0.3
1	Cit.	20	27	4.7	8.9	15	24	0.12	0.17	.3	
2	Cit.	4	20	3.4	8.9	3.0	12		0.22	.4	

P'_i = Apparent inorganic phosphorus; P_o = organic phosphorus; P_t = total phosphorus; Pyr = pyruvate; α keto = alpha-ketoglutarate; Lact = lactate, Cit = Citrate.

FAT METABOLISM

As balance measurements have shown, fat absorption is abnormally low, although with variations according to the geographic region in which the studies have been performed (25, 34, 51). No difference has been detected between typical kwashiorkor and other cases of malnutrition (25, 51). As the nutritional status improves, fat absorption is better but generally does not reach normal levels. There is a direct correlation between absolute intake and absorption of fat ($r = +0.89$), which suggest the convenience of not restricting fat intake in severely malnourished children. On normal feedings, most of the fecal fat consists of free fatty acids (9) while in children fed only parenterally, the greatest proportion of fat excretion is neutral fat.

Concentrations of total lipids and cholesterol are abnormally low in children with kwashiorkor. There is a quick rise once treatment is started (11, 55, 56). Similar findings have been made in regard to alpha-lipoproteins (11). A definite prognostic significance has been attached to the rise in cholesterol; its absence is apparently indicative of unfavorable conditions for recovery (55). These changes in serum lipid fractions are similar in magnitude and time relationships, to those observed in total lipids, total cholesterol and lipoproteins, during the development of normal children during the 1st year of their life (41). Thus, this would be another evidence in favor of the structural and biochemical resemblance of the malnourished child with subjects of a younger age.

EXTRA AND INTRACELLULAR WATER

Also in this aspect, the malnourished child is in a certain way very similar to the normal newborn. Dilution has been mentioned as one of the universal signs of malnutrition, and has been demonstrated by dye and isotope dilution studies (19, 20, 26, 65), and direct tissue analyses (15). The malnourished child shows an increased plasma and total extracellular fluid volume, and overall overhydration of his body tissues (31), both intra- and extracellularly, regardless of the presence or absence of pitting edema, or of clinically evident 'dehydration.' There seems to be an arrest in the tendency to dessiccation which characterizes normal growth in the human being (16). In regard to solutes, depletion of body potassium has been regularly found (15, 32, 62, 65), although this is not necessarily reflected in serum concentrations. Magnesium and phosphorus have also been found reduced in actual amounts and intracellular concentration. Dynamic changes related to clinical decline or death have been characterized as a still greater expansion of intracellular fluid phase, with further decrease of potassium and increase of sodium intracellular concentrations. At the same time, there is a considerable decrease of organic phosphorus fractions, and an increase of inorganic and readily available organic phosphorus. These changes have been interpreted in terms of the relationships with specific intermediary metabolites, on the premise that the ion serves as an activator or inhibitor in a given enzymatic sequence (39). Death from kwashiorkor seems to be associated with a striking reversal of the sodium to pyruvate and of inorganic and organic phosphate to pyruvate ratios in the cell, these changes being consistent with some defect in the K, Mg and P-dependent formation of pyruvate. Similar changes are observed in the ratios of Mg, inorganic phosphorus and citrate to alpha-ketoglutarate, suggesting an inhibition in the common

metabolic cycle. This might be consistent with the unusual amino acid patterns, fatty livers and persistence of subcutaneous fat which characterizes kwashiorkor in spite of gross caloric deficiency. These data are shown in table 6; the ratios were obtained on values expressed per unit of noncollagenous nitrogen, observed on admission, and 7–10 days later in children who improved, or at the moment of death in those who died.

ENZYMATIC CHANGES

The data described above point toward anomalies in the enzymatic systems of the malnourished organism. So far, extensive studies indicate that in the systems studied (3, 6, 13, 63, 66), the most affected enzymes are those in which the physiological function is either unknown, or of little transcendency; it is noteworthy that cytochrome reductase is very little modified, even in children with apparent riboflavin deficiency. On the other hand, working with more complex enzymatic systems, whose activity depends upon integrity of liver mitochondrias, it has been found that in malnutrition respiration and oxidative phosphorylation are maintained; however, the systems involved seem to have a greater liability to ageing or heat, in malnourished livers than in normal ones (67). Further information is needed in this aspect.

TREATMENT

Three basic points have been outlined in regard to treatment of malnutrition in childhood, whatever the severity of the same, or the clinical appearance (43): *1*) it should tend towards the elimination of the etiologic factors involved; *2*) it should permit repairing of the damage caused; *3*) it should be sufficiently prolonged to obtain rehabilitation.

Thus, universal signs of malnutrition as such will initiate a regression as soon as an appropriate treatment is started; the same may be said of circumstantial signs. Aggregated manifestations, mostly signs of an agression superimposed on previous malnutrition, deserve an emergency treatment, whose main phases will be focused on the acute electrolyte disturbance, and on intercurrent infection.

In dealing with acute electrolyte disturbances, and once the state of hypovolemia has been urgently corrected with the usual resources, it has to be taken into account that the severely malnourished child has become adapted to a lowered osmolality of his body fluids, which will prevail as long as the child remains malnourished, viz., keeps a subnormal rate of growth and development. It has been ascertained, that when parenteral fluid administration becomes necessary, the ideal solution for these children is one isotonic with their own blood plasma, which generally, whether in acute disturbance or not, has an osmolality ranging from 230 to 280 mOs/l. Specific deficiencies are so far being replaced only in regard to potassium and calcium, but experience is being obtained with the use of phosphate buffer solutions and magnesium salts.

Regarding infection in malnourished children, an exhaustive review has shown that the dominant interaction between nutrition and infection is in the sense of a synergism (59). Very frequently, subjects showing a deficient status of nutrition become infected, sometimes with a microorganism regarded as nonpathogenic, or are overcome by infections ordinarily not fatal. Multiple infections, larger infecting doses and other environmental conditions may be part of the explanation. Children with advanced malnutrition are at high risk of fatal infection; our experience shows that infection is demonstrated in 45% of the children admitted to the hospital, and in most of them it has been the event that brings on the acute episode which motivates hospitalization. Most frequently, the diagnosis is difficult to establish, since more often than not, the symptomatology is strikingly atypic and of little intensity (50). Frequently, one can rely only on data derived from peripheral blood cell counts, immunological reactions, and x-ray studies.

Under these conditions, results of treatment are frequently erratic, and failures seem to be in direct relation to the deficiency of nutritional status.

Once the acute episode is controlled, dietary management of the severely malnourished child does not differ from the principle which is valid in any type of chronic primary malnutrition. The subject is given a normal diet, generous in calories and with an adequate proportion of natural proteins of high biological value; the food should be given in a way which makes easy its intake, pleasant, easy to digest, and in amounts determined by the free demand of the patient. It should be adapted to the taste of the subject, which will be related to certain tissue requirements and to his cultural background.

Anorexia, mostly due to emotional causes, may interfere partially with this scheme. However, good pediatric care by a well-trained nursing personnel generally helps to overcome this phase.

According to the metabolic data mentioned before, at no stage is there any indication for restriction in fat intake. There is no need for additional vitamins especially of those which act as cofactors in normal metabolic sequences, since they will be needed simultaneously with their substratum. Sometimes it is convenient to limit the rapid loss of excessive edema fluid, providing a supplement of salt.

Once anorexia has disappeared, intake rapidly increases, and the response of the organism may be an accurate index of the specific needs of different organs of the body. Hypochromic anemia and rickets are among the nutritional complications to be looked for, and to be treated with accepted methods if they occur.

The intake of as much as 5 g of good protein and up to 300 Cal/kg of body weight, in children with chronic advanced malnutrition, generally gives rise to a quick resumption of growth, usually at an exaggerated speed, which eventually will give place to the picture known as Nutritional Recovery Syndrome (27). There are no essential differences in this picture, as shown by children originally affected with kwashiorkor or marasmus (49).

PREVENTION

It may be postulated that (10): *1*) malnutrition in childhood, as commonly seen in technically underdeveloped countries, starts around the 4th month of extrauterine life; *2*) it seems to be related to lack of consumption of foodstuffs necessary to cover the requirements of the growing organism, at a time when mother's milk becomes insufficient; *3*) withholding additional food may be related, in a great number of cases, to the 'fear towards food,' which probably has arisen from secular experiences derived from the impact of very poor environmental sanitation, maintained by the predominance of magic and premicrobian concepts about the etiology of disease and supported very often by professional prescription; *4*) as the child grows older, his participation in adult's diet becomes progressively greater; *5*) at all ages, nutritional status will be conditioned by food availability, particularly in countries which do not have an agricultural policy tending mainly to the production of food, and where land production is mainly destined to exportation; where communications are insufficient and a great part of the best crop is lost because of poor storage facilities; *6*) the importance of the problem of childhood malnutrition is seldom recognized in an integral fashion; interest in the matter is generally focused on the terminal picture, that is, on circumstantial and aggregated signs, on the 'accident' which throws the child into a state of emergency; much less attention is given to the universal signs, ever present where inadequate nutrition induces a decrease in the rates of growth and development; *7*) as far as the affected population is concerned, since lack of adequate growth is so generalized, no abnormality in this sense is usually detected or complained of; on the other hand, the 'accident' is practically never related to nutrition, but, as stated before, it is mostly attributed to magic influences and its cure considered beyond a physician's capacity; *8*) although it is true that when economic conditions improve, the bases for popular beliefs tend to be weakened, it must not be forgotten that for most of the people, food and medicine are completely different things, and that it is much more difficult to obtain acceptance of concepts of disease prevention based on sufficient nutrition, than faith derived from the prescription of a medicament.

It may be surmised, that unless shaped to these facts, health education programs aimed at improving communal nutritional status, will make slow, if any, advances.

A related approach, somewhat different in its essence, is the idea of interrupting the vicious circle of: sequelae of infant malnutrition plus inadequate feeding in the adult → diminished working capacity → poor productivity → decreased food disponibility. One of the solutions proposed in this regard is the provision of mixtures of vegetable proteins; the factors to be taken into account in this kind of project have been described in detail (4, 12). The main usefulness of these mixtures may be as substitutes for the materials presently used on a wide scale for the preparation of thin cereal gruels, which in Mexico and Central America are practically the only foods used for babies before and during weaning.

Another solution of the problem consists in the supplementation of low-quality food with synthetic amino acids. It has been shown (28) that supplementing a diet of corn meal and beans with lysine and tryptophane, improved the absorption and retention of nitrogen in 4 children with chronic advanced malnutrition; similar results were obtained by matching the amino acid composition of a corn diet given in amounts corresponding to 3.0 (60) and 1.5–2.0 (5) g of protein/kg body weight to 3 boys recovering from severe protein malnutrition, with that of the FAO 'reference protein.' More exhaustive study seems necessary, before this measure can be recommended on a wide scale.

At the present moment, it seems that the introduction of a good full source of animal protein into foodstuffs of common daily use, without changing their physical characteristics, or interfering with the traditional nutritional habits of the population, may be a satisfactory temporary measure. Tests on the acceptability of such supplemented foods, by normal and malnourished subjects, and on the biological value of poor diets supplemented with totally deodorized fish flour, have given encouraging results (29). Experience is being accumulated in regard to acceptance and consumption of these foods by large population masses, under the prevailing poor conditions of communal, familial and personal sanitation.

It must be emphasized that any preventive program should have, as a common base, not to interfere with the basic culture of the community, but rather to understand it, classifying the prevailing practices into beneficial, innocuous and harmful ones; only after that, one may intend the elimination of the latter, by means of methods similar to those which historically acted in the community and gave birth to present uses (10). This implies the initiation of further projects whose objective is to obtain this kind of knowledge.

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Adult protein requirements

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Since Voit stated in 1881 that a certain amount of protein is required to be taken daily to maintain healthy life, many studies have been performed to estimate the protein requirement from various standpoints. From the survey of the amount and kind of food eaten by people, predecessors of nutritionist, Voit (1881), Atwater, Rubner, etc., concluded that some 120 g of protein are necessary per day for an adult person of 70 kg body weight.

On the other hand, Siven (1901) was able to maintain himself in nitrogen equilibrium on about 0.4 g of protein per kg per day. Many of his supporters, i.e. Hindhede, Chittenden, etc. whose experiments warranted the low protein requirement appeared after him. A controversy between these 2 groups lasted for many years and it might be said in some respects that the controversy still continues today.

There seems to be a general agreement at present that a daily intake of 0.5 g/kg of body weight is sufficient for the adult to maintain his nitrogen balance (24), and the recent experiments of Rose (22) as well as those in Stare's laboratory (11) revealed that about 0.35 g/kg/day should be enough (28), if the quality of protein is good enough to provide a safe level of all the essential amino acids.

On the other hand, since Folin's work (1905), it is generally recognized that the protein balance may be maintained with a low-protein intake by adaptation, i.e. by reducing the reserve protein in the body, and by decreasing the rate of protein catabolism (2, 36). Therefore, it is a matter of dispute whether or not such a low-protein intake as a minimum to maintain the protein balance may be considered adequate to satisfy physiological need.

Whipple (29) emphasized an important rôle of reserve protein in the body and many physiologists and physicians (1, 3, 20, 36) presented evidences indicating that a certain amount of protein store is necessary to ensure good health, especially to resist stress.

Probably by taking a surplus intake over the minimum protein requirement into consideration as a desirable factor of safety, the League of Nations recommended in 1935 that the protein intake for all adults should not fall below 1 g of protein per kg of body weight. From advances in knowledge accumulated during 20 years thereafter, the FAO Committee of Protein Requirement (9) adopted a new approach to estimation of protein requirement. They defined, first, a reference protein of high nutritive value in order to relate the protein requirement to its quality, and adopted 0.35 g/kg/day as the minimum requirement of reference protein for maintenance of balance in the adult. In calculating the safe practical allowance from the protein requirement, they recommended to allow for individual variation and for quality of the protein contained in diet, and abandoned to take account of reserve protein, while they justified adopting a liberal approach in allowing for protein reserve.

How to explain and evaluate the difference between the protein requirement and the practical allowance for protein, i.e. the safe intake, is the problem which is the most important but most unclarified in the present knowledge of protein requirement. The following are reviews and criticisms from the author's works on those factors governing the protein requirement of the adult human and its related problems.

MINIMUM PROTEIN REQUIREMENT

Minimum protein requirement to be discussed here is the smallest amount of protein which can maintain nitrogen balance in the adult when diet is adequate in other respects. It is well known, however, that there is a number of limitations for determining protein requirement. Sherman (24) emphasized that the most influential factor for the minimum requirement is the extent to which the subject has become accustomed to a low-protein intake. Allison pointed out that the minimum requirement determined by the balance technique is influenced by the nutritional status of the individual (2). The requirement measured with a protein-depleted dog is lower than that with a nondepleted one and thus variety of physiological status of subject may lead to divergence of opinion regarding requirements. Harper (10) reviewed recent knowledge of protein and amino-acid requirements, and emphasized that the most important thing in this field of study is to establish some basis for reporting requirements so that the values presented are relatively little influenced by various factors.

These physiological and experimental limitations for estimation of maintenance requirement were ascertained by human experiments in the author's laboratory (mostly not yet published in English) of which examples are illustrated in figures 1, 2 and 3.

Figures 1 and 2 are a part of human experiments

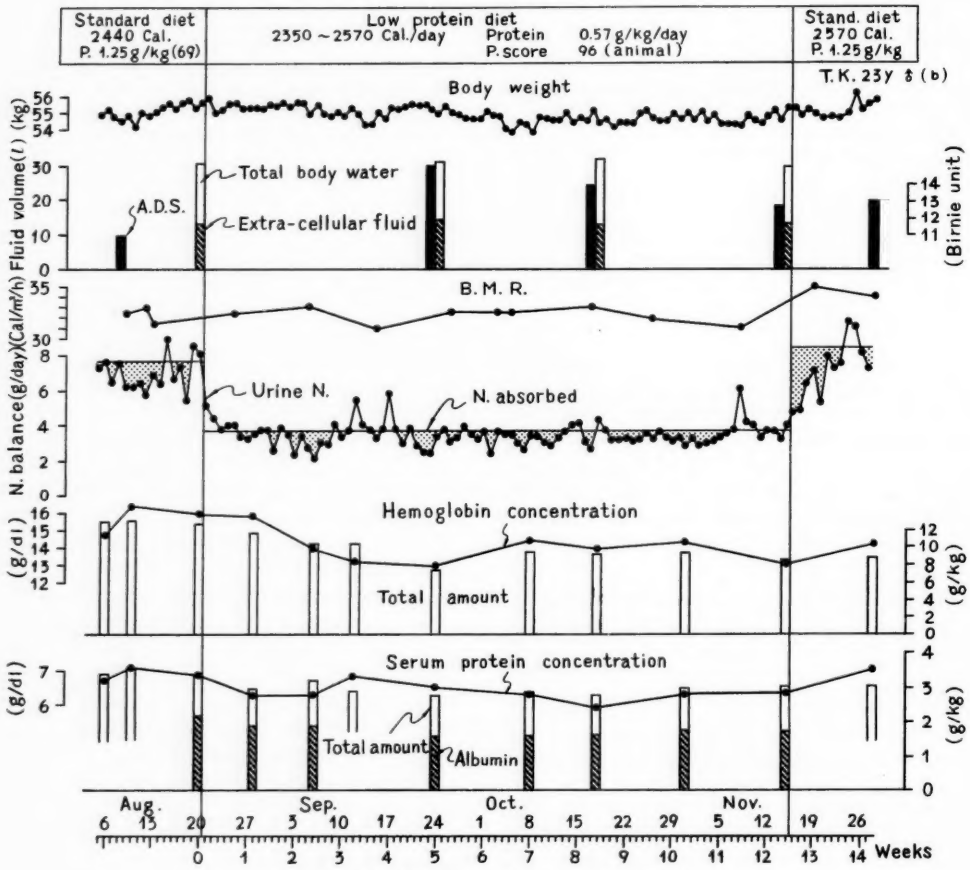

FIG. 1. Physiological effects of low protein diet (1).

which were performed to see physiological effects of low protein diet. The amount of protein intake in the experiments in 2 figures was almost the same, i.e. about 0.57 g/kg/day, but its quality is quite different. The dietary protein in figure 1 was mainly of animal origin (protein score 96), while that in figure 2 was mainly composed of cereal protein. Limiting amino acids of the latter were tryptophane and methionine and the protein score was 63. The caloric content of diet was enough to fulfil the energy requirement, and vitamins and minerals were sufficiently provided by a certain supplement. The subjects had been nourished with a standard diet containing 1.2–1.5 g/kg protein (30% animal protein) for a week or longer prior to low protein diet, and their nutritional states were standardized. As demonstrated in the figures, nitrogen balance attained the zero level in a week with low protein diet of good quality, while it took about 2 weeks in the case of poor nutritive protein. Thus the quality of dietary protein can influence nitrogen balance.

Calculations of nitrogen balance were made in the 2nd week as well as in the 3rd week of low protein diet in 11 experiments with male adults (22–42 hr.), and are plotted against protein intake in figure 3. Dietary protein is vegetable in origin (largely rice protein) and its protein score is 60–73. A good correlation exists in each of 2 sorts of graphs, and the slope of regression line, i.e. the nitrogen balance index for total nitrogen intake, is about 0.35 for the 2nd week and 0.43 for the 3rd week. This means that availability of dietary protein to nitrogen balance is improved as the adaptation to low protein diet proceeds (2).

The points at which regression lines cross the line of protein intake give the average minimum protein for maintaining the zero balance in the 2nd and the 3rd week of low protein diet, respectively. The value thus obtained corresponds to the minimum protein requirement, and is 0.086 g nitrogen per kg, corresponding to 0.54 g protein per kg per day, when the balance was calculated from results in the 3rd week, while it is 0.097 g nitrogen per kg from data of the 2nd week. Thus, by longer adaptation, protein metabolism is reduced to maintain balance with lower protein intake. The time allowed to attain nitrogen balance is the most important factor in estimating the minimum protein requirement.

With the metabolic nitrogen being taken as 0.6 g/day by experiments, the balance for absorbed nitrogen was calculated, and is illustrated in the same figure. The absorbed nitrogen for zero balance is 0.072 g/kg, corre-

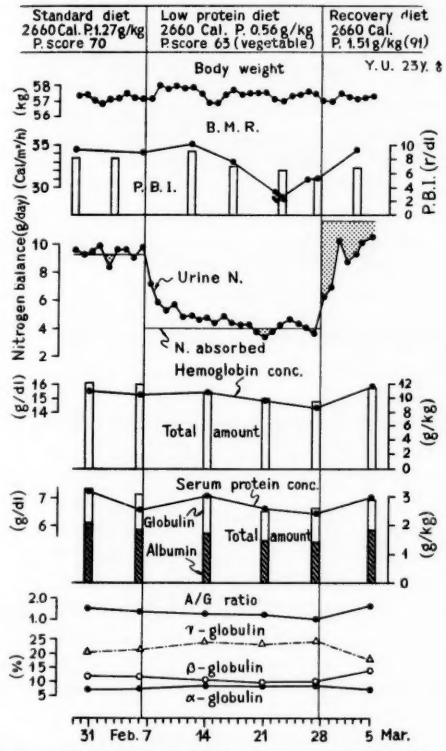

FIG. 2. Physiological effects of low protein diet (2).

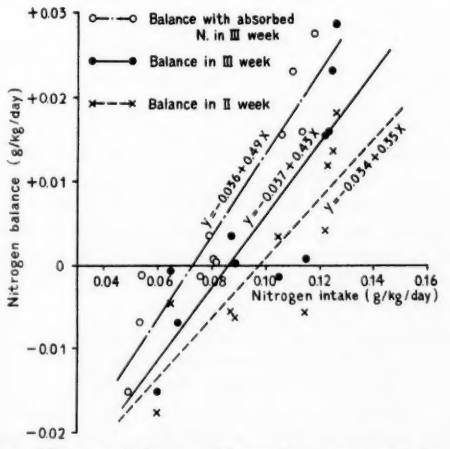

FIG. 3. Nitrogen balance with various amounts of protein intake.

sponding to 0.45 g/kg/day protein which is completely digestible, and the balance index is 0.49.

Hindhede (12) reported an experiment of low protein diet of long duration lasting over half a year. The protein intake which was sufficient to maintain the zero balance was 0.3 g/kg/day. The diet was composed mainly of potato and fruits, and thus its protein quality was not high. He emphasized in his paper that the subjects could subsist on the diet. Nevertheless, the photograph of the subject in his paper gives an impression of edematous appearance, and data of blood properties indicated anemia. With longer experiment, nitrogen balance might be maintained with lower protein level, but protein malnutrition finally appears. Thus, the minimum protein requirement, estimated from the nitrogen balance in experiments lasting too long seems to be somewhat dangerous. The most important thing is to find the protein requirement which is capable of maintaining normal physiological functions.

Have previous data of protein requirement been subjected to criticism on this point? Recent advances in studies on physiological effects of low protein diet present valuable knowledge in answering the question, and will be discussed in the following section.

PHYSIOLOGICAL EFFECTS OF LOW PROTEIN INTAKE

Physiological effects of protein deficiency have been extensively studied in connection with famine occurring during and after the war, and from this knowledge, the widespread existence of malnutrition and disease due to protein deficiency have been recognized (4, 23, 27). Researches on protein malnutrition have thrown new light on the problem of protein requirement, and it has come at present to the forefront of interest among nutritionists. Frequent symposiums and conferences have been held to deal with these problems (2, 9, 10, 25, 28).

The crux of the problem is how to evaluate the optimum requirement. The optimum requirement here indicated is the requirement which is sufficient to maintain normal physiological functions, and is not very different from the safe intake or practical allowance.

In an attempt to explain the difference between the protein requirement and the optimum requirement, the author studied physiological effects of low protein intake in human adults (male) with special reference to protein balance. Following are outlines of experimental results from the author's laboratory (37).

Figures 1 and 2 are typical examples which clearly demonstrate physiological effects of low protein intake, even just over the protein requirement. As seen in figure 1, nitrogen balance was maintained within a week after the beginning of a low protein diet of 0.57 g/kg which is good in quality. Nevertheless, erythrocyte count and hemoglobin content in 100 ml blood as well as in total circulating blood began to decrease in the 2nd week. Anemia was somewhat, but not completely, improved after 6 weeks or so, and a light anemia persisted even after 3 months (cf. fig. 6). Serum protein content showed a similar decrease as hemoglobin. The ratio of albumin to globulin decreased significantly, and consequently the decrease in serum protein resulted mainly from reduction of albumin content (cf. fig. 2 and fig. 6). On the other hand, the fraction of γ-globulin showed a tendency to increase. Leukocyte count also decreased in association with relative lymphocytosis. These changes in blood properties will be discussed in detail under a separate article.

The volume of body fluid, especially that of extracellular fluid increased in protein deficiency, and a slight edema frequently appeared. Body weight fluctuated and this fluctuation was associated with variation of urine volume. As caloric intake was adjusted so as to be increased with the rate of 3% for 10°C lowering of environmental temperature, the fluctuation of body weight seemed to be largely due to that of water balance.

Similar facts are illustrated in figure 2, which is a result of low protein intake of poor quality (0.56 g/kg). A distinct difference from that in figure 1, however, can be seen in the course of basal metabolism which began to fall in 2 weeks, while it was kept almost constant for about 3 months in figure 1.

As nitrogen balance arrived at the zero level a week later than that in the case of good protein (fig. 1), nitrogen loss during the period of negative balance was larger in the case of low protein quality, and this larger nitrogen loss may be mainly responsible for a remarkable reduction in basal metabolic rate (BMR).

Urobilinogen in urine appeared shortly after a low protein diet, indicating an impairment of liver function. With animal experiments, reduction in activities of various enzymes in the liver were verified to be reduced. Among these, cathepsin, arginase, catalase, xanthine dehydrogenase, xanthine oxidase, etc. have been studied from an early period (16, 18, 30, 35). These changes in enzyme activities are supposed to be an indication of their wearing due to protein depletion, and contribute to decrease protein catabolism in adapting to low protein intake.

There are many evidences that low protein intake may also influence hormonal activities, even when nitrogen balance is well maintained. These will be discussed under a separate heading.

Anemia in protein deficiency. Striking effects of protein deficiency are reduction of hemoglobin and serum albumin as was already mentioned. Serum albumin is known as a metabolizable protein, and thus its reduction may result from being utilized in protein metabolism. On the other hand, hemoglobin is contained in erythrocyte and is not so easily utilized. Consequently the cause of hemoglobin reduction in protein deficiency has been a matter of dispute (27, 29, 31).

To elucidate the nature of anemia, hematocrit, erythrocyte diameter and osmotic resistance of red cells in NaCl solution were measured in association with erythrocyte count and hemoglobin determination in the above mentioned experiments. From these measurements, color index, volume index, mean corpuscular volume (M.C.V.), mean corpuscular diameter (M.C.D.) and mean corpuscular thickness (M.C.T.) were calculated (31). Figure 4 is an illustration of data with 4 subjects who took 0.6–0.8 g/kg of protein daily for 3 months. The color index and the volume index decrease somewhat after 1 month of protein deficiency but return to the normal level after the 2nd month. A similar change is seen in M.C.D. in the figure. Therefore, anemia is hypochromic-microcytic in the early period but later it

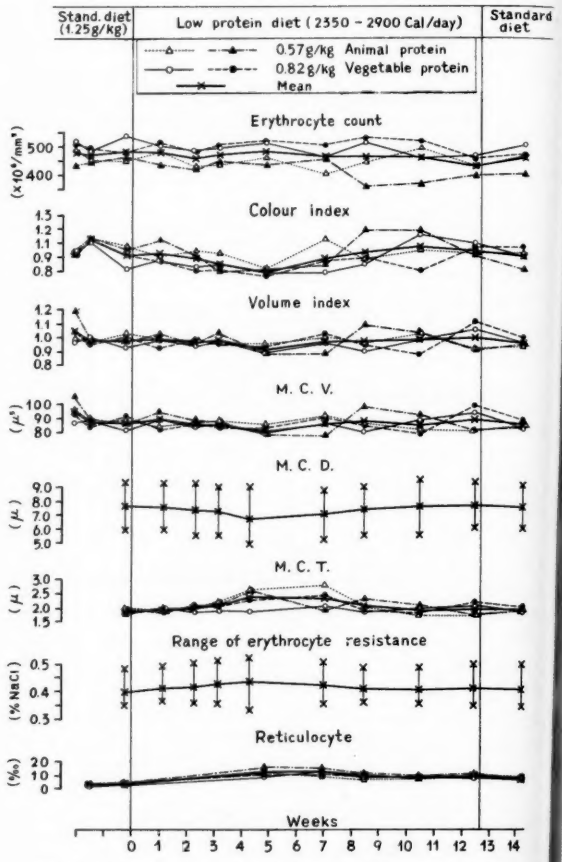

FIG. 4. Anemia in protein deficiency. (Refer to note 1.)

becomes normocytic. As M.C.T. increases toward the end of the 1st month, some degenerative process is suggested to have appeared in erythrocyte membrane, and the suggestion is supported by the fact of decreased osmotic resistance in these periods. The degenerative process of membrane may facilitate destruction of erythrocyte, thus leading to anemia.

This hypothesis is supported by various evidences as follows: reduction of total hemoglobin content amounts to 14% after 2 weeks' protein deficiency on the average. As the average life of human erythrocyte is believed to be 110 days (31), this amount of reduction cannot be explained only by an assumption of retarded regeneration due to protein deficiency. On the other hand, reticulocyte count increases in association with growing anemia (fig. 4), and thus regeneration of erythrocyte is actually accelerated. Consequently, some destructive process of erythrocyte should be responsible for the anemia.

As an important cause of erythrocyte destruction, depression of metabolic activity of erythrocyte is suggested, because recent investigations revealed that resistance of erythrocyte membrane is maintained by its metabolic activity (19), and protein deficiency may

provoke a wearing of enzyme system in red cells as is generally evidenced in other organs.

Hormonal activities in protein deficiency. In the Fourth International Congress on Nutrition, Roch (21) spoke of the influence of malnutrition upon sexual hormones in depressing their secretion. According to him, clinical signs and pathologico-anatomic findings indicate their hypofunctions. In chronic malnutrition, urinary excretion of 17-ketosteroid was verified to decrease. Inoue (13) examined histologically various endocrine organs taken from cadavers of severe malnutrition, and verified degenerative changes in pituitary gland, adrenal gland, thyroid gland, Langerhans' islet, testicle, ovary, etc.

From these studies, the author (not yet published) started to examine activities of various endocrine glands in experiments of low protein diet. A part of the results are illustrated in figure 5 which presents daily excretion of 17-OHCS and 17-KS in urine during the experimental period. Amount and quality of protein taken in this period are those indicated in figure 1 (0.57 g/kg protein of high quality) for *cases a* and *b*, and 0.82 g/kg protein of low quality (protein score 67) for *cases c* and *d*. Nitrogen balance is well maintained throughout the experimental period of 3 months except for several days in the beginning stage. Excretion of 17-OHCS as well as of 17-KS decreases day by day until 1 month or so, then is restored gradually and finally attains a constant level somewhat below the original after 1½ months. The reduction of excretion of steroids evidences decrease of adrenocortical function.

As seen in figure 1, activity of antidiuretic substance (ADS) in serum determined with Birnie's method increases in protein deficiency, and may be responsible for the increase of extracellular fluid or edema mentioned above. A cause of increase of ADS may be an impairment of liver function due to protein deficiency, because liver is known to inactivate ADS and it was ascertained by animal experiments that increase of ADS activity in serum in protein deficiency can be partly prevented by administration of liver protecting agents such as glucuronic acid, methionine and a mixture of riboflavin and pantotenic acid (17).

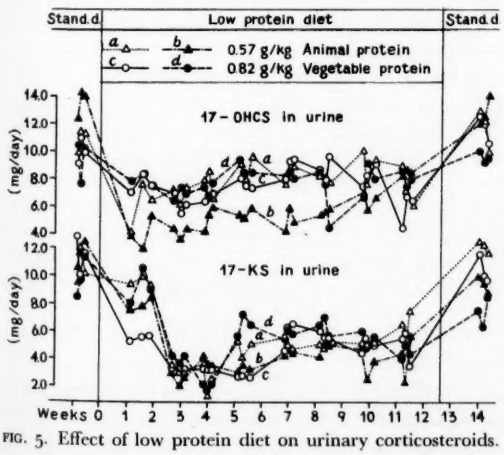

FIG. 5. Effect of low protein diet on urinary corticosteroids.

Protein bound iodine (PBI) in serum also undergoes a slight reduction which parallels that of BMR already mentioned (fig. 2). Thus a decrease of thyroid activity is effected by protein deficiency and may cause reduction of BMR. Affection of thyroid gland seems to be achieved only after a large loss of body protein due to protein deficiency because it is not apparent in figure 1 in which loss of body protein is less than that in figure 2.

It should be mentioned here that nitrogen balance was well maintained in these experiments. Thus hormonal activities undergo disturbances only by taking protein in lower quantity than a certain limit.

Although causes of these hormonal disturbances are not well known, it is reasonable to suppose that they are associated with the wearing of enzyme systems which control metabolism of hormones, as is verified with enzymes in liver and others. These changes in hormonal activities will reflect upon disturbances of physiological functions which should be studied in more detail in future.

APPROACH TO OPTIMUM REQUIREMENT

As mentioned above, physiological properties and functions of various organs undergo changes by taking a protein deficient diet, even when protein balance is maintained. Impairment of physiological functions may be attributed to loss of reserve protein in the body. The reserve protein is a highly metabolizable protein in the body and is the most likely to be lost by low protein intake. Loss of reserve protein is accompanied by disintegration of the enzyme system which results in metabolic depression of important organs. Decreased metabolism necessarily induces impairment of physiological functions.

Besides for maintaining the physiological functions, a certain amount of protein store is needed for permitting resistance to stress. Yamamoto verified in the author's laboratory that reduction of basal metabolism due to protein depletion is retarded by a preliminary protein repletion (35). Chiba demonstrated by experiments with rabbits that healing of a skin wound is promoted by protein repletion prior to injury (7). Protein intake which guarantees an adequate quantity of reserve protein is nothing but the optimum requirement.

In order to evaluate the optimum requirement, it is necessary to answer the following questions:

1) What is the measure of adequate protein store?

2) How much and what sort of protein is necessary for maintaining the normal level of protein store?

3) What influence may physiological state or stress exert on the optimum requirement?

As a tentative measure of adequate protein store, it is reasonable to take either the total hemoglobin or the total albumin or both in circulating blood, because there is much evidence that blood protein including hemoglobin acts as reserve protein by being utilized after disintegration in protein deficiency (29, 34).

In an attempt to evaluate the optimum requirement with this measure, Yamamoto (34) in the author's

laboratory, determined previously blood protein content of many individuals in association with their daily protein intake. The hemoglobin content decreased below the normal range, as the protein intake was lower than 1 g/kg/day, while it fluctuated in the normal range when the intake was more than 1 g. Unfortunately, however, the measurement was made at that time only on the concentration of hemoglobin and on that of serum protein. These concentrations may sometimes be influenced erroneously by water content in blood. On the other hand, there is other evidence which proves protein intake of 1 g/kg to be a limit to maintain normal blood protein level. Buddhist monks who are taking 0.9–1.3 g/kg of vegetable protein (mainly cereal and pulse) may frequently show the normal total hemoglobin and plasma protein contents. From human experiments with low protein diet, however, the author ascertained that 0.8 g/kg protein of vegetable origin (protein score 67) may not be sufficient to maintain the normal level of total hemoglobin content in circulating blood (see *c* and *d* curves in fig. 6). Therefore, it is reasonable to take about 1 g/kg protein as the minimum amount for maintaining the normal level of blood protein for a certain group when dietary protein is largely a vegetable one.

In order to cover individual variations, this amount seems to be raised a little higher. On the other hand, most of the Japanese diet contains some animal protein (about 30% of the total protein on the average). With protein of better quality, the value may be lessened. The Committee on Protein Requirement in FAO (9) presented a formula by which the requirement of reference protein can be converted to that for the protein actually contained in the diet by allowing for its protein score. As the premise for the formula, it is assumed that the protein requirement is inversely proportional to the protein score. In order to verify this assumption, a series of human experiments were performed.

Two sorts of dietary protein, i.e. one of good quality (protein score 96) which is mainly animal in origin and the other of poor quality (protein score 67) which is entirely of vegetable origin, were prepared for examination. The amount of intake was 0.57 g/kg for the former, and 0.82 g/kg for the latter. Thus, intakes of these 2 sorts of protein are just inversely proportional to their protein scores. The experimental diet was taken for 3 months by 4 adult male subjects, and physiological effects were examined. Figure 6 is a part of results which illustrate changes of total hemoglobin and total albumin contents in circulating blood expressed as the percentage of their respective controls before experiment. It is clear in the figure that physiological effects of protein deficiency are more serious with lower intake of animal protein (*a* and *b* in fig. 6) than those with vegetable protein (*c* and *d*). Thus, these 2 sorts of protein intake are by no means of the same nutritive value, and an increase of protein intake seems to raise the nutritive value more than to cover the lack in protein quality. As the amounts of protein have been calculated in both cases so as to just fulfill the requirements of essential amino acids prescribed by FAO, the amount of limiting amino acids are the same, while the quantity of non-essential amino acids is larger in the vegetable diet than in the animal one. Therefore, it is reasonable to conclude that the larger amount of low qualified protein is better than the lower amount of high qualified protein under certain circumstances.

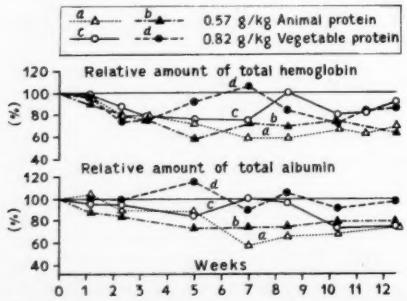

FIG. 6. Percentage changes of total hemoglobin and serum albumin contents in circulating blood during the period of protein deficient diet.

In any case, the FAO formula for conversion of quality of protein to quantity is incomplete and cannot be utilized in the strict sense for evaluation of the optimum requirement, and requires more extensive studies. From these arguments, however, it seems reasonable to take the optimum requirement to be 1 g/kg/day for a population of individuals, when the quality of protein is adequate.

In order to arrive at a safe practical allowance for a nation, the optimum requirement should be adjusted to allow for individual variations as well as variations of dietary form. In the present situation in which various unknown factors remain unsettled, the Committee of National Nutrition in Resources Council in Japan (8) has provisionally adopted 0.5 g/kg/day as the average minimum requirement of protein supplied in the habitual Japanese diet in well digestible form. It corresponds to the value obtained by converting the requirement of reference protein, 0.35 g/kg, to that of protein in the national diet (protein score 70) by the FAO formula ($0.35 \div 0.70 = 0.5$). Moreover, it is in accord with experimental results obtained with Japanese diet which was described above.

The coefficient 2.0 has been preferred to 1.5 of FAO as a safety factor allowing for individual variation of protein requirement as well as an adequated guarantee of reserve protein. By taking the wide variety of digestibility of the Japanese diet into consideration, another factor 1.25 has been adopted to allow for digestibility of dietary protein. All these factors being taken in calculation, 1.25 g/kg of protein ($= 0.5 \times 2 \times 1.25$) is recommended for adults as a practical allowance for Japanese.

PROTEIN NEED AT VARIOUS PHYSIOLOGICAL STATES, ESPECIALLY IN HEAVY LABOURING

As is well known, there are various physiological states at which additional protein is required over the normal maintenance in the adult age. Among these, pregnancy,

lactation, and heavy labouring are important. Protein requirements during pregnancy and lactation were fully dealt with in the Conference at Princeton, U. S. A. (28), as well as by the Committee of FAO (9). The present author studied protein needs in heavy labouring for a number of years, and will discuss this problem in the following.

Since Atwater (cited from Sherman (24)) stated that muscular work sufficient to double the energy metabolism has no appreciable effect upon the amount of protein metabolism, no special concern has been paid to the protein need in heavy labouring by various countries, including U. S. A. On the other hand, Cathcart (6) insisted that protein metabolism is really promoted in muscular work, though the nitrogen balance might not be changed because influences of acceleration of both catabolism and anabolism cancel each other.

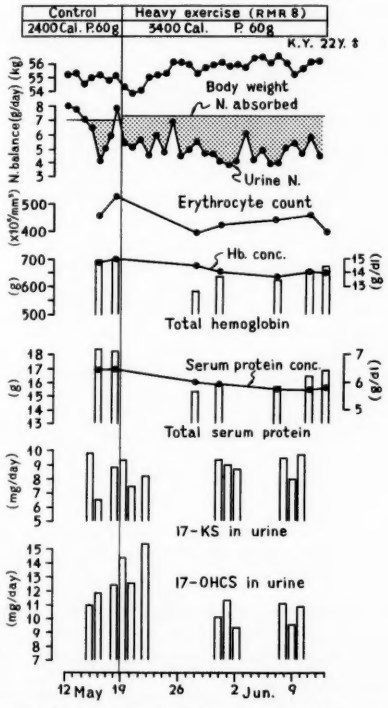

FIG. 7. Physiological effects of long continued heavy muscular exercise. (Refer to note 2.)

Yamaji (33) and Yoshimura *et al.* (38) studied the protein metabolism during a long continuing heavy muscular exercise, with students as subjects (19–24 yr.), and found that protein balance was changed to positive by decreased nitrogen output. An example is illustrated in figure 7. Muscular exercise was performed with a bicycle ergometer for 2.2 hr. daily, and its relative metabolic rate (RMR = energy requirement/BMR) was 8, corresponding to energy requirement of 1000 Cal/day. The dietary protein was maintained constant near the level of optimum requirement in a resting state, while calories were raised to meet the increased energy metabolism. Vitamins and minerals were sufficiently supplied.

Notwithstanding positive protein balance, anemia and hypoproteinemia appear after about 10 days training (fig. 7). This anemia has been proved to be due to destruction of erythrocyte (5, 32), and is accompanied with acceleration of red cell formation in bone marrow which is indicated by remarkable increase of reticulocyte. Consequently, the development of anemia ceases after 2 or 3 weeks in an ordinary case. Anemia does not appear in the subject who has already been trained to exercise, because anemia has been restored by this increased blood cell formation.

Hiramatsu (not yet published) followed the fate of radioactive iron tagged by hemin in erythrocyte which is introduced into exercised rats. Hemin iron of transfused blood was found to accumulate preferably in skeletal muscles when animals were being trained to strenuous exercise. Thus, it is evidenced that hemoglobin from destroyed erythrocyte may be utilized for hypertrophy of skeletal muscle in the training subject. Reduction of plasma protein, especially of its albumin fraction,

TABLE 1. *Dietary Protein and Blood Properties in Training to Muscular Exercise*

Dietary Protein		Body Weight of Subj., kg	Heavy Exercise		Mean Nitrogen Balance, g/day	Total Hemoglobin Content, g		Total Serum Protein, g	
g/kg/day	Animal protein, %		R.M.R.	Duration (days)		Before ex.	Change after ex.	Before ex.	Change after ex.
1.0	19.7	50.3	8.0	15	−0.04	710	−90	176	−25
1.0	25.7	58.4	8.0	25	+1.45	675	−66	194	−39
1.1	25.7	55.1	8.0	25	+2.31	701	−33	183	−14
1.2	23.2	50.1	9.0	20	+0.11	711	−95	176	−25
1.2	24.9	50.7	7.3	21	−0.24	610	−100	156	−21
1.4	24.4	53.1	8.0	15	+0.42	693	−79	157	−6
1.9	41.6	53.2	8.5	21	+1.67	565	+1	157	+1
1.9*	57.4	64.3	8.0	27	+2.80	727	−2	182	+5
2.3	23.3	51.0	9.0	21	+1.69	610	+9	156	+13
2.4	26.5	50.0	7.9	21	+3.46	634	+106	153	+20
3.0	57.3	50.3	9.0	15	+3.78	630	+90	153	+20

* Pure casein supplement.

may also be explained by its being utilized in such a promotion of tissue building. Tendency of tissue growth in organs working in muscular exercise leads necessarily to acceleration of the positive balance of nitrogen. Thus, protein need is increased during the training of muscular exercise. Kraut *et al.* (15) verified a decrease of basal metabolism when the subject who took 1 g/kg/day of protein with sufficient caloric content, was loaded daily with heavy muscular work for some months. Moreover, the training effect on muscular activity was not as good as when excess protein was provided (14). Consequently, they emphasized an increased protein need during long continuing heavy muscular work, though they could not estimate an exact amount.

Usami (26) verified an increase of protein catabolism during strenuous muscular exercise by measuring non-protein nitrogen in blood. He explained the increase as being due to stress by heavy work, because urinary output of 17-OHCS was increased by heavy muscular exercise. This increase of 17-OHCS secretion appears only at the beginning stage in the training course (stage of alarm reaction according to Selye), and secretion of 17-KS gradually increases in its place in the later stage (stage of resistance) (fig. 7). The increase of 17-KS in the later stage may promote protein synthesis and may be another factor contributing to positive nitrogen balance.

General tendency of tissue destruction due to increased protein catabolism in the beginning of muscular training may play a rôle in the destruction of erythrocyte, because this destruction is especially remarkable in the early stage. Thus anemia appearing with strenuous muscular training seems to be rather physiological in origin.

It was demonstrated by experiment that anemia and hypoproteinemia due to muscular exercise may be prevented by taking excess protein in diet. With increased protein intake, consumption of serum protein is saved, and regeneration of erythrocyte is accelerated to cover reduction of hemoglobin due to red cell destruction. As dietary protein, protein of high quality such as casein (even in a pure form), meat and fish protein is more effective than cereal protein. Effects of various amounts of dietary protein upon anemia and hypoproteinemia in the training period of physical exercise are presented in table 1.

From the results, it is concluded that about 2 g/kg/day of protein may suffice to prevent reduction of blood protein, including hemoglobin, during the physical training, provided that animal protein supplied is about 25% of the total dietary protein.

The author's thanks are due to Prof. K. Kodama for his encouragements for these works.

NOTE 1. Read the scale of erythrocyte count in fig. 4 as one hundredth of the printed figure. For example, 500×10^6 should be read as 500×10^4.

NOTE 2. In fig. 7, the scale of erythrocyte count should be read as one hundredth of the printed figure. The figures on the scale of total serum protein should be read ten fold as large as the printed figures. For example, 13 g should read as 130 g.

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Panel Summary—Proteins and Amino Acids

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A BRIEF SUMMARY of nearly two hours of questions and answers can describe only a few of the highlights. The first major area of discussion centered about the problem of protein reserves and, in contrast to some previous meetings, there was unanimous agreement that, whether or not the name is entirely appropriate, 'protein reserves' do exist and are important; they are actually the tissue proteins in the cells of the more labile tissues. These tissue proteins, including enzymes, when broken down contribute to the amino acid pool.

Dr. Patwardhan suggested that the loss in protein malnutrition of considerable quantities of both riboflavin and nitrogen in the urine, and the decrease of riboflavin-dependent enzyme activity in the liver as well as the simultaneous accumulation of riboflavin and protein upon repletion, support this concept. Dr. Allison emphasized the primary use of relatively dispensable protein such as that of skeletal muscle, parenchymatous organs, and serum albumin for protein reserves and the limited contribution of cardiac muscle and nervous tissue.

Dr. Yoshimura cited evidence that haemoglobin breakdown can contribute amino acids for the synthesis of the muscle under conditions of physical training. I pointed out that marasmus is an extreme example of utilization of protein reserves, and that perhaps kwashiorkor represents a loss of ability to utilize amino acids from tissue breakdown for the resynthesis of essential proteins.

The next major area of discussion was the characteristics of protein deficiency. It was not possible to go into these with any degree of completeness, but the overhydration of the cells which characterizes protein deficiency was emphasized by both Dr. Frenk and Dr. Patwardhan. They suggested that in the refeeding of protein depleted individuals, excess water leaves the cells and nitrogen re-enters. This would help to explain the frequent failure to observe a weight change during early treatment of kwashiorkor even with a strongly positive nitrogen balance. The full explanation of this phenomenon is, however, still unknown.

Dr. Frenk emphasized the sensitivity of growth failure as an indicator of protein deficiency in children and also the usefulness of urinary nitrogen excretion patterns in both children and adults. Dr. Brock expressed the opinion that in adults, serum albumin values in the range of 3·5–2·7 g/100 ml are indicative of early protein deficiency.

It seemed probable to Dr. Brock that in parts of Africa a causal association exists between inadequate diets, the frequency of cirrhosis of the liver and the subsequent development of hepatic carcinoma. I questioned the significance of this association because kwashiorkor is very common in Central America and yet cirrhosis of the liver is rare and primary liver carcinoma almost unknown.

The discussion then centered around a number of practical considerations. The concept of an optimum dietary amino acid pattern to meet over-all body needs was presented and defended by Dr. Allison with the qualification that appropriate allowance must be made for the digestibility of the ingested protein and the amino acid pattern actually reaching the blood stream. He cautioned, moreover, that different physiological and pathological states and individual tissues and enzyme systems have amino acid requirements which differ from the over-all pattern. The panel noted that the ratio of protein to total calories is also of great importance in determining the adequacy of a given protein intake and the consequences of an apparent protein deficiency.

There was a discussion of the relative merits of animal and vegetable proteins, and the panel emphasized that protein quality is not measured by the origin of the protein but by its ability to meet amino acid needs. They agreed that as long as the required pattern of essential amino acids is supplied, the extent to which it comes from animal, vegetable and even synthetic sources should make no difference to the body; an almost infinite number of food combinations can meet these needs. Several of the panelists called attention to the fact that it may be practical, under certain circumstances, to supplement foods with synthetic amino acids in order to achieve an improved amino acid pattern.

Dr. Autret stressed the global importance of protein malnutrition in this 'protein decade' as the Chairman referred to it in his introduction. Dr. Autret and the other members of the panel considered it important to meet the problem of protein malnutrition by developing all practical sources of protein including fish and fish flours, oil seed press cakes, pulses and legumes as well as milk, meat, eggs and cheese. They noted that technically underdeveloped countries require help in food technology as well as in agricultural production and the improvement of dietary habits. All agreed on the need to increase awareness of protein needs and to insure the necessary production and consumption of protein-rich foods in the technically underdeveloped areas.

PANEL III

Lipids in Health and Disease

Chairman: Ancel Keys, USA
Moderator: K. Lang, Germany

Stoffwechsel der lipide

K. LANG

Physiologisch-chemisches Institut der Johannes Gutenberg-Universität Mainz, Mainz, Germany

Die stellung der lipide in der Ernährung des Menschen und damit der Lipidstoffwechsel stehen immer mehr im Mittelpunkt des Interesses. Es ist daher verständlich, daß in den letzten Jahren ein ungeheures, in seinen Details kaum mehr von einem einzigen Forscher überschaubares experimentelles Material zusammengetragen wurde. Trotzdem bestehen auch heute noch große Lücken unseres Wissens und viele mit der ernährungsphysiologischen Bedeutung der Lipide zusammenhängenden Fragen lassen sich im gegenwärtigen Augenblicke nicht oder nur unbefriedigend beantworten. Es wird daher als eine wichtige Aufgabe dieses Referates betrachtet, auf solche Lücken hinzuweisen, wobei in erster Linie Fragen behandelt werden sollen, die mit den ernährungsphysiologischen Eigenschaften der Lipide zusammenhängen.

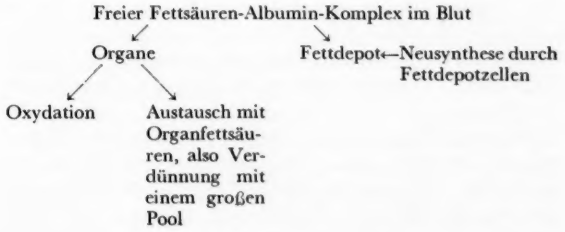
Die Problematik fängt schon bei dem Mechanismus der Resorption der Lipide an. Das bei der Resorption beteiligte Transportsystem (vermutlich hydrophiler Art), das bei der Aufnahme der mehr oder minder tief durch die Verdauungsenzyme aufgespaltenen Lipide in die Mucosazellen ist weitgehend unbekannt. Das Phänomen der cocancerogenen Wirkung von Detergentien zeigt eindringlich, wie wichtig hier eine Vertiefung unserer Kenntnisse wäre. Ebenso wäre eine Vertiefung der Kenntnisse im Hinblick auf die Resorptionsmöglichkeiten von chemisch veränderten Fettsäuren, insbesondere polymeren Fettsäuren wünschenswert.

Das resorbierte Lipidmaterial wird von den Mucosazellen an die Lymphe in Form von Chylomikromen abgegeben. Diese bestehen zum größten Teil aus Triglyceriden, in dem Cholesterin und Cholesterinester gelöst sind, umgeben von einer stabilisierten Schicht von Phospholipiden und Proteinen. Die Relation der einzelnen Bestandteile kann in gewissem Umfange schwanken. Das Verhältnis Phospholipoidfettsäure: Triglyceridfettsäuren wird offensichtlich etwas von der Art der verfütterten Fettsäure beeinflußt.

Die Chylomikronen erscheinen im Blut und bedingen dort eine Reihe von Phänomenen, die im Zusammenhange mit der Entstehung von ischämischen Kreislauferkrankungen eine erhöhte Beachtung gefunden haben wie Verkürzung der Blutgerinnungszeit und das Sludge-Phänomen. Offensichtlich erfahren oberflächenbedingte Eigenschaften der Erythrocyten während der Lipämie Veränderungen, die sich u.a. auch in einer Erschwerung des Gasaustausches äußern und daher Aufmerksamkeit verdienen.

Die Chylomikronen behalten im Blut ihre charakteristischen Eigenschaften bei und die in ihnen enthaltenen Lipide beteiligen sich nicht an Austausch- oder Stoffwechselprozessen bis zu ihrer Zerstörung, die in den Geweben, hauptsächlich in der Leber stattfindet. Bei diesen Prozessen ist eine Lipoproteidlipase beteiligt, deren Abgabe durch Heparin stimuliert wird. Unsere Kenntnisse hierüber sind aber noch lückenhaft und die Frage, ob die Heparin-Clearing-Reaktion die einzige für das Verschwinden der Chylomikronen aus dem Blut verantwortlich zu machende Reaktion ist, läßt sich noch nicht eindeutig beantworten.

Die aus den Chylomikronen in Freiheit gesetzten Fettsäuren werden entweder in den Organen rasch oxydiert oder sie erscheinen wieder im Blut teils in Form von Lipoproteiden hoher Dichte, teils als freie, an Albumin gebundene Fettsäuren. Letztere haben im Blut eine Halbwertszeit von nur wenigen Minuten. Ihr Schicksal zeigt das Schema:



Welcher Weg in einem konkreten Falle bevorzugt beschritten wird, hängt von der augenblicklichen Stoffwechselsituation ab. Bei Hungertieren überwiegt die Oxydation, bei reichlich mit Kohlenhydrat gefütterten die Ablagerung im Depotfett. Fettsäuren mit 24 und mehr C-Atomen werden kaum mehr an Albumin gebunden. Die Trägheit ihres Stoffwechsels dürfte damit zusammenhängen.

Bekanntlich speichern Mensch und Tier alles über den Bedarf zugeführte, als Calorienspender verwertbare Material in Form von Fett. Die Speicherung erfolgt in Zellen, die erhebliche Mengen an Triglyceriden

aufnehmen können. Beim Menschen besteht das gespeicherte Fett zum Teil aus dem Nahrungsfett, wobei zumeist nur geringfügige Umwandlungen von Fettsäuren beobachtet werden, zum Teil aus von den Fettgewebszellen synthetisiertem Fett. Das Depotfett ist demnach beim Menschen—im Gegensatz zum Wiederkäuer—in seiner Zusammensetzung variabel und es ist nicht uninteressant, daß beim Menschen offensichtlich keine physiologische Notwendigkeit besteht, ein konstant zusammengesetztes Depotfett zu besitzen.

Die calorische Verwertung der Nahrungsfette setzt ihre Oxydation im intermediären Stoffwechsel voraus. Der Abbau aller Fettsäuren vollzieht sich prinzipiell durch β-Oxydation, bei der aus den geradzahligen Acetyl-CoA, aus den ungeradzahligen daneben auch noch Propionyl-CoA entsteht.

Für die Aktivierung der Fettsäuren durch Bindung an Coenzym A sind bisher drei verschiedene Thiokinasen nachgewiesen worden: die Azetatthiokinase (Substratbereich vorwiegend C_2 und C_3), die Oktanatthiokinase (Substratbereich vorwiegend C_4-C_8) und die Dodekanatthiokinase (Substratbereich C_5-C_{22}, maximale Geschwindigkeit mit C_{12}). Der Reaktionsmechanismus ist für alle drei Enzyme derselbe.

Neben der Aktivierung der Fettsäuren durch ATP spielen noch Transferreaktionen eine Rolle wie z.B.

Acetyl-CoA + Propionsäure $\rightleftarrows$ Propionyl-CoA + Essigsäure.

Außer Propionsäure können noch viele andere normalen Fettsäuren das Coenzym A von dem Acetyl-CoA übernehmen.

Die an das Coenzym A gebundene Fettsäure durchläuft einen vierstufigen Cyclus, bei dem 4 H-Atome abgegeben werden und die un 2 C-Atome ärmere, an Coenzym A gebundene Fettsäure entsteht. Im Falle der Stearinsäure mit ihren 18 C-Atomen wird dieser Cyclus achtmal durchlaufen. Dabei werden 32 H-Atome frei und es entstehen 9 Mole Acetyl-Coenzym A. Die H-Atome dienen auf dem üblichen Wege der biologischen Oxydation durch Verbrennung zu Wasser der Gewinnung von Energie.

Bei dem Fettsäurecyclus sind 4 Enzyme bzw. Enzymarten beteiligt. Die Dehydrierung der gesättigten Acyl-CoA-Verbindungen erfolgt durch die Acyldehydrogenasen. Sie sind Flavinenzyme. Bisher sind drei verschiedene Acyldehydrogenasen nachgewiesen worden, die Butyryldehydrogenase (Aktivität von C_2-C_8), die Capryldehydrogenase (Aktivität von C_4-C_{16}, Optimum mit C_8) und die Lauryldehydrogenase, die gegen die höheren Fettsäuren eine größere Aktivität aufweist als die Capryldehydrogenase.

Die einziege bisher nachgewiesene Enoylhydratase (Krotonase) hat ein breites Wirkungsspektrum. Sie hydratisiert sowohl α,β-ungesättigte Säuren als auch β,γ-ungesättigte Säuren und wirkt vermutlich auch als cis-trans-Isomerase.

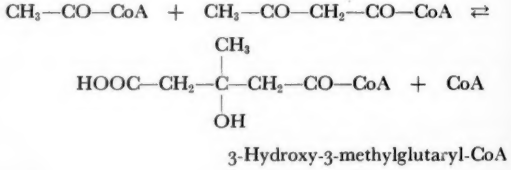
Das nächste Enzym im Fettsäurecyclus, die β-Oxyacyldehydrogenase, hat ebenfalls ein weites Wirkungsspektrum und setzt alle Säuren von C_4-C_{18} bei völliger Substratsättigung mit etwa derselben Geschwindigkeit um. Bei den Hydroxybuttersäuren ist die Dehydrogenase specifisch auf L(+)-Hydroxybuttersäure eingestellt. In tierischen Geweben wurde jedoch eine Racemase nachgewiesen, die L(+)-β-Hydroxybutyryl-CoA wechselseitig in D(−)-β-Hydroxybutyryl-CoA überführt. Außerdem enthalten Lebermitochondrien noch eine spezifisch auf D(−)-β-Hydrocybutyryl-CoA eingestellte Dehydrogenase. Die β-Hydroxyacyldehydrogenase ist ein mit DPN arbeitendes Pyridinenzym.

Bisher ist nur eine einzige β-Ketothiolase bekannt geworden.

Das Auftreten großer Mengen Acetessigsäure beim Diabetes mellitus hat schon frühzeitig das Interesse auf den Mechanismus der Bildung dieser Substanz gelenkt. Die Aufklärung desselben erfolgte jedoch erst in der jüngsten Zeit. Die Vorstufe der Acetessigsäure ist das Acetoacetyl-CoA, das beim Abbau der Fettsäuren durch β-Oxydation im Fettsäurecyclus auf der Stufe der C_4-Säuren entsteht oder aber auch durch Kondensation von 2 Molekülen Acetyl-CoA gebildet wird.

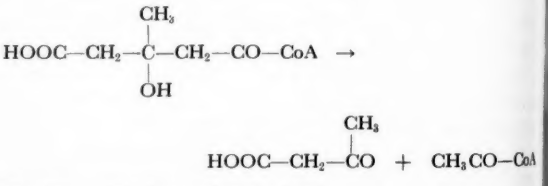
Die Freisetzung von Acetessigsäure aus dem Acetoacetyl-CoA erfolgt in der Leber durch einen Prozeß, dem β-Hydroxy-β-methylglutaryl-CoA-Cyclus.

Acetoacetyl-CoA wird durch ein der Leber enthaltenes Enzym ("Enzym B") mit Acetyl-CoA zu 3-Hydroxy-3-methylglutaryl-CoA kondensiert:



3-Hydroxy-3-methylglutaryl-CoA

Dieses wird dann durch das HMG-CoA-cleavage enzyme in Acetessigsäure und CoA aufgespalten:



Die Bilanzgleichung des Cyclus ist:

2 Acetyl-CoA + H_2O $\rightleftarrows$ Acetessigsäure + 2 CoA.

Die in der energiereichen S-Acylbindung in dem Acetoacetyl-CoA enthaltene Stoffwechselenergie geht hierbei verloren. Die Bildung freier Acetessigsäure in der Leber ist daher eine praktisch irreversible Reaktion. Hierauf ist die schon lange bekannte Tatsache zurückzuführen, daß die Leber zwar imstande ist, große Mengen an Acetessigsäure zu bilden, diese aber nicht zu oxydieren vermag.

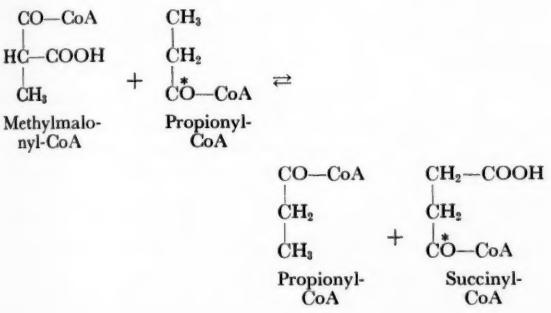
In der Niere und Muskulatur, die—wie man schon seit längerer Zeit weiß—Acetessigsäure zu "verbrennen" vermögen, erfolgt die Freisetzung von Acetessigsäure aus dem Acetoacetyl-CoA durch einen anderen Prozeß, bei dem die Energie in Form von ATP erhalten bleibt:

Acetoacetyl—CoA + Succinat ⇄ Succinyl-CoA + Acetoacetat
Succinyl-CoA + ADP + P ⇄ Succinat + CoA + ATP

Summe: Acetoacetyl-CoA + ADP + P ⇄
Acetoacetat + CoA + ATP

Durch die Umkehrung dieser Reaktionskette wird der Abbau der freien Acetessigsäure eingeleitet.

Der Organismus baut auch Fettsäuren mit einer ungeraden C-Atomzahl durch β-Oxydation ab. Dabei entsteht, neben viel Acetyl-Coenzym A, zuletzt auch Propionyl-Coenzym A. Dieses wird unter CO_2-Fixierung über Methylmalonyl-CoA und Succinyl-CoA in Bernsteinsäure übergeführt. Die enzymatische Isomerisierung des Methylmalonyl-CoA zum Succinyl-CoA erfolgt durch eine Transcarboxylierung:



Bei der Co_2-Fixierungsreaktion wird ein "aktiviertes" CO_2 verwendet. Die Aktivierung von CO_2 erfolgt durch ein von BACHHAWAT und COON kristallisiertes Enzym. Das aktivierte CO_2 wird dann auf geeignete Acceptoren übertragen wie z.B. Propionyl-CoA oder β-Hydroxyisovaleryl-CoA, wobei im letzteren Falle 3-Hydroxy-3-methylglutaryl-CoA entsteht. Freie Propionsäure wird im Organismus zuerst durch die Acetat-thiokinase aktiviert.

Die Uberführung von Propionsäure in Succinat macht die alten Befunde, daß der tierische Organismus Propionsäure leicht oxydieren oder zur Bildung von Glucose bzw. Glykogen verwenden kann, ebenso die Beobachtung, daß der C^{14} von C^{14}-Propionsäure in verschiedenen Aminosäuren (Alanin, Glutaminsäure, Leucin, Prolin und Glykokoll) gefunden wird, leicht verständlich.

Das bei der β-Oxydation im Fettsäurecyclus entstehende Acetyl-Coenzym A wird dann entweder durch Kondensation mit Oxalacetat der Endoxydation über den Citronensäurecyclus zugeführt oder zu Biosynthesen verwendet.

Obwohl sich der Abbau der niederen und mittleren Fettsäuren im intermediären Stoffwechsel prinzipiell gleich wie der der höheren durch β-Oxydation vollzieht, so ergeben sich doch zwischen ihnen und den letzteren wesentliche Unterschiede im Stoffwechsel und auch in der ernährungsphysiologischen Bedeutung.

Bei der Biosynthese der Fettsäuren in den Zellen des tierischen Organismus entstehen keine niederen und mittleren Fettsäuren, mit der einen Ausnahme: der Milchdrüse. Daher ist das Milchfett reich an diesen Fettsäuren.

Die niederen Fettsäuren haben noch eine gewisse, bei den niedersten Gliedern sogar sehr große Wasserlöslichkeit. Schon dieser Unterschied in ihrem physikalischen Verhalten beeinflußt ihre physiologischen Eigenschaften beträchtlich. Sie werden daher auch nicht wie die höheren Fettsäuren nach der Resorption durch die Lymphe sondern durch die Pfortader vom Darm transportiert und müssen daher zwangsläufig die Leber passieren. Die Einbaugeschwindigkeit der niederen und mittleren Fettsäuren in Triglyceride und Phosphatide ist außerordentlich klein, da die Geschwindigkeit des Acyltransfers vom Coenzym A auf Glycerophosphat erst ab einer C-Atomzahl von etwa 14 genügend groß wird. Die niederen und mittleren Fettsäuren werden daher weder im Depotfett noch in den Organen gespeichert und fallen wegen der fehlenden Veresterung einen raschen Abbau anheim. Der Rest wird zu allerlei Biosynthesen über das intermediär entstehende Acetyl-Coenzym A verwendet. Durch die rasche Umwandlung wird die Konzentration der niederen Fettsäuren im Blut normalerweise außerordentlich nieder gehalten. Erhöht man sie durch i.v. Injektion der Salze oder Triglyceride solcher Fettsäuren, so ergibt sich eine relativ hohe Toxicität, die in gesetzmäßiger Weise mit der C-Atomzahl zusammenhängt und ihr maximum bei der Capronsäure mit einer LD_{50} von 120 mg/kg Körpergewicht für Tricapronin bei der Maus erreicht. Symptome sind Somnolenz bis zu tiefer Bewußtlosigkeit, Blutdrucksenkung und Bradycardie. Solche Befunde sind naturgemäß im Zusammenhange mit dem Problem der parenteralen Ernährung durch intravenöse Infusion von Fettemulsionen von Bedeutung.

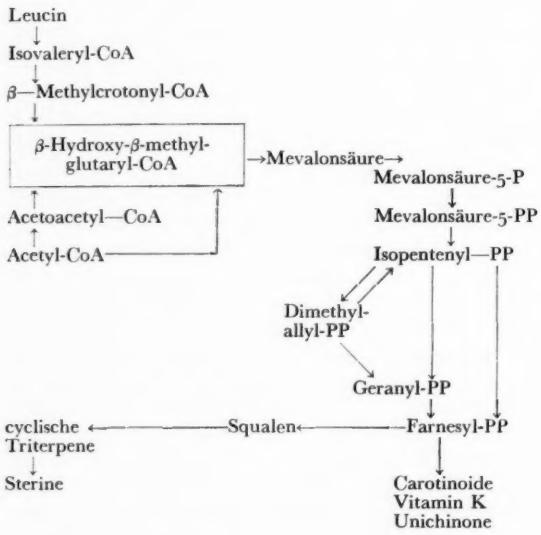
Die Frage nach dem Abbau verzweigtkettiger Fettsäuren hat im Zusammenhange mit dem Stoffwechsel gewisser, von Bakterien gebildeter Fettsäuren und dem einiger Aminosäuren (Valin, Leucin, Isoleucin) ein größeres Interesse. In der neueren Zeit wurde das Vorkommen verzweigter Fettsäuren in tierischen Fetten festgestellt. Weiterhin ließ sich zeigen, dass verzweigte Fettsäuren Zwischenprodukte bei der Biosanthese von Sterinen und anderen Isoprenderivaten sind.

Das Verhalten der alkylierten Fettsäuren im Stoffwechsel wird durch die folgenden Faktoren bestimmt: *1*) Zahl der C-Atome und sterische Anordnung der Seitenkette; *2*) Zahl der C-Atome der Hauptkette; *3*) Stellung der Verzweigung zur Carboxylgruppe; *4*) Zahl der Verzweigungen und ihre gegenseitige Lage; *5*) etwaiges Vorkommen quaternärer C-Atome. Bei Fett-

säuren mit einer langen Hauptkette werden die Seitenketten besser abgebaut als bei Fettsäuren einer mittleren C-Atomzahl. Je weiter die Carboxylgruppe von der Seitenkette entfernt ist, umso schlechter wird die Verbrennlichkeit der Substanz im Organismus. Mit zunehmender Länge der Seitenkette wird der Abbau immer mehr erschwert.

Der oxydative Abbau von Leucin liefert Isovaleriansäure, die nach Aktivierung und CO_2-Fixierung über β-Hydroxy-β-methylglutaryl-CoA zu Acetyl-CoA und Acetoacetat abgebaut wird.

β-Hydroxy-β-methylglutaryl-CoA ist Substanz von großer physiologischer Bedeutung, da sie das Bindeglied zwischen Fettstoffwechsel und der Biosynthese von Sterinen und Isoprenderivaten ist:



Die 1929 gemachte Entdeckung, daß Mensch und Tier einige im intermediären Stoffwechsel zu bestimmten Zwecken benötigte Fettsäuren nicht selber zu bilden vermögen und daher auf die Zufuhr dieser Fettsäuren mit der Nahrung angewiesen sind, war der erste Hinweis, daß nicht alle die Nahrungsfette zusammensetzenden Fettsäuren dieselbe ernährungsphysiologische Bedeutung haben. Die beiden wichtigsten dieser, als "Essentielle Fettsäuren" bezeichneten Substanzen sind

All-cis-$\Delta^{9,12}$-Oktadekandiensäure (Linolsäure) und
All-cis-$\Delta^{5,8,11,14}$-Eikosatetraensäure (Arachidonsäure).

Die essentiellen Fettsäuren haben im Stoffwechsel bestimmte Sonderaufgaben. Voraussetzung für ihre physiologische Wirkung ist neben der All-cis-Konfiguration die Atomgruppierung —CH=CH—CH_2—CH=CH—, also das Vorhanden sein einer aktiven Methylengruppe zwischen zwei Doppelbindungen, die außerdem noch an einer bestimmten Stelle der C-Atomkette und zwar gerechnet vom Beginn der Kohlenstoffkette (CH_3—) an den C-Atomen 6 und 9 stehen muß.

Aus Linolsäure wird unter Verlängerung der C-Atomkette und Einbau neuer Doppelbindungen im Divinylmethan-Rythmus Arachidonsäure gebildet. Zwischenprodukt ist aller Wahrscheinlichkeit nach γ-Linolensäure ($\Delta^{6,9,12}$-Oktadekantriensäure), von der bekannt ist, daß sie die physiologische Wirkung einer essentiellen Fettsäure besitzt. Dagegen ist Linolensäure ($\Delta^{9,12,15}$-Oktadekantriensäure) kein Zwischenprodukt.

Linolensäure geht im tierischen Organismus in fünffach und sechsfach ungesättigte Säuren mit 20 bzw. 22 C-Atomen über.

Beim Mangel an essentiellen Fettsäuren häuft sich im Organismus eine Triensäure an, die als $\Delta^{5,8,11}$-Eikosatriensäure identifiziert wurde und nicht die physiologischen Eigen schaften einer essentiellen Fettsäure besitzt und auch deine Wachstumswirkung entfaltet. Sie entsteht aus Ölsäure durch Verlängerung der C-Atomkette und weiterer Dehydrierung.

Die Fischöle enthalten etwa 70–80% Polyensäuren jedoch nicht vom Typus der essentiellen Fettsäuren; sie tragen daher nicht wesentlich zur Versorgung des Menschen mit essentiellen Fettsäuren bei. Die wichtigsten in den Fischölen aufgefundenen Polyensäuren sind:

$\Delta^{6,9,12,15}$-Hexadecantetraensäure
$\Delta^{6,9,12,15}$-Octadecantetraensäure
$\Delta^{5,8,11,14,17}$-Eicosapentaensäure
$\Delta^{1,7,10,13,16,19}$-Docosahexansäure.

Die Stoffwechselreaktionen der Polyensäuren lassen nach dem Gesagten verschiedene Reaktionsketten und verschiedene Typen von Polyensäuren erkennen, die im Stoffwechsel unabhängig voneinander sind, unterschiedliche physiologische Wirkungen entfalten, und nicht miteinander verwechselt werden dürfen.

Über den Wirkungsmechanismus der essentiellen Fettsäuren ist man nur mangelhaft unterrichtet. Beobachtungen über die Beeinflussung von Enzymaktivitäten in den Substrukturen der Zellen durch den Mangel an den essentiellen Fettsäuren weisen darauf hin, daß diese Fettsäuren vielleicht bei der Bindung von Enzymen an die Strukturen und überhaupt bei dem Aufbau von Strukturen eine Rolle spielen.

Der Organismus hat nur eine beschränkte Fähigkeit zur Speicherung von essentiellen Fettsäuren. Sind die Speicher gefüllt, so wird die über den Bedarf zugeführte Linolsäure im Stoffwechsel oxydiert und nur noch calorisch verwertet. Die Geschwindigkeit des Verbrauchs ist vom Lebensalter abhängig und bei jugendlichen Individuen größer als bei älteren. Die Speicherung der essentiellen Fettsäuren erfolgt—abgesehen vom Depotfett—bevorzugt in bestimmten Organen, vor allem in Leber und Niere, in geringerem Umfange in Herz und Milz. Das Nervensystem ist dabei praktisch unbeteiligt.

Über den Abbaumechanismus der essentiellen Fettsäuren ist man gegenwärtig nicht unterrichtet, ebensowenig über den der anderen Polyensäuren.

Im Zusammenhange mit dem Problem der Hydrierung von Speisefetten ferner ihren anaeroben oder oxydativen Polymerisierungen ist die Frage nach dem Stoffwechsel von Polyensäuren mit konjugierten Doppelbindungen von Bedeutung geworden. Die vorliegenden Untersuchungen zeigen, daß solche Polyensäuren resorbiert, in den Organen gespeichert und auch im Organismus abgebaut werden. Näheres über den dabei beteiligten Reaktionsmechanismus ist jedoch nicht bekannt. Besser orientiert ist man über den Stoffwechsel der Sorbinsäure, der konjugierten Hexadiensäure, die nach Aktivierung wie die entsprechend gesättigte Säure im Fettsäurecyclus oxydiert wird.

Bei den essentiellen Fettsäuren wirken nur die All-cis-Formen als solche. Die cis-trans- oder die All-trans-Formen sind biologisch inaktiv. Auch die in der Natur aufgefundenen Monoensäuren liegen zumeist ausschließlich in der cis-Form vor. In der neueren Zeit sind jedoch im Körperfett, ferner auch im Milchfett von Wiederkäuren trans-Fettsäuren aufgefunden worden. Sie verdanken ihre Entstehung der Hydrierung von Polyensäuren durch die Mikroorganismen im Pansen. Der Gehalt der Butter an trans-Fettsäuren beträgt 4–11 % bezogen auf die Gesamt fettsäuren. Auch bei der katalytischen Härtung der Fette entstehen trans-Fettsäuren. Margarine enthält 3–20 %. Bei Nicht-wiederkäuern werden trans-Fettsäuren im Körperfett nur nach Verfütterung solcher aufgefunden. Im Depotfett des Menschen machen sie im Mittel 12 %, im Leberfett 14 % der gesamten Fettsäuren aus. Bei der Ratte gehen trans-Fettsäuren nicht von der Mutter auf den Fetus über. Reichert man einen Organismus durch Verfütterung reichlicher Mengen an trans-Fettsäuren an und setzt dann die Zufuhr an solchen ab, so verschwinden die trans-Säuren wieder aus den Organen. trans-Fettsäuren werden demnach im Organismus umgesetzt. Auch Versuche in vitro ergaben, daß trans-Säuren abgebaut werden und zwar in der prinzipiell gleichen Weise wie die cis-Fettsäuren. Ein Anhaltspunkt, daß die trans-Fettsäuren im Organismus toxisch wirken, hat sich nicht ergeben. Die Ermöglichung des Abbaues der trans-Säuren ist vermutlich durch die als cis-trans-Isomerase wirkende Crotonase bedingt.

In der neueren Zeit wurde von verschiedenen Autoren gezeigt, daß dem Fett im Blut und in den Organen regelmäßig kleine Mengen an Fettsäureperoxyden beigemischt sind. Der tierische Organismus verfügt zwar über keine Lipoxydase. Es ist aber schon seit längerer Zeit bekannt, daß Häminenzyme eine der Lipoxydase analoge Wirkung in geringem Umfange entfalten können. Es ließ sich auch an isolierten Mitochondrien die Bildung von Fettsäureperoxyden, kenntlich am positiven Thiobarbiturattest nachweisen. Diese Befunde verdienen ein großes Interesse sowohl im Zusammenhange mit der Tocopherolwirkung als auch mit dem Problem der Toxicität oxydierter Fettsäuren. Über das Schicksal von Fettsäureperoxyden im Stoffwechsel ist man nicht unterrichtet.

Ein Referat im Rahmen eines Ernährungskongresses muß naturgemäß in erster Linie auf die Verwertung einer Substanz im Stoffwechsel d.h. auf die Abbau- und Umbaumechanismen abgestellt sein. Daher soll die Biosynthese der Fettsäuren nur kurz gestreift werden.

Daß der tierisch Organismus zur Biosynthesee von Fett befähigt ist, war schon lange bekannt. Hinweise auf den Reaktionsmechanismus haben sich jedoch erst in der neueren Zeit auf Grund von Versuchen mit markierten Substraten gewinnen lassen. Versuche an Tieren, deren Körperwasser mit D_2O angereichert war, hatten ergeben, daß sich die Biosynthese der Fettsäuren aus Acetat bzw. "aktiviertem Acetat" (Acetyl-CoA) vollzieht und im Prinzip eine Umkehrung der β-Oxydation darstellt. Hauptorte der Biosynthese von Fett im Organismus sind Leber und Darmwand. Grundsätzlich sind jedoch alle Gewebe zu dieser Leistung befähigt. Einen besonders hohen Umfang der Fettsäuresynthese findet man in der lactierenden Schilddrüse. Bei der Durchströmung eines lactierenden Kuheuters mit Acetat-1-C^{14} können 40 % und mehr des C^{14} in dem sezernierten Milchfett gefunden werden.

Die bei der Fettsäuresynthese beteiligten Enzymsysteme lassen sich in Lösung bringen. Es war daher in der neueren Zeit möglich, die Biosynthese von Fettsäuren in vitro an einfachen, wasserlöslichen Systemen zu studieren. Die meisten Untersuchungen wurden an Enzymsystemen der Rattenleber, Taubenleber oder der Milchdrüse angestellt. Interessanterweise verläuft die Fettsäurebiosynthese in diesen 3 Systemen nicht völlig identisch, wenngleich die zu beobachtenden Unterschiede nicht groß und keineswegs prinzipieller Art sind.

Bei der Biosynthese von Fettsäuren durch hochgereinigte Enzymsysteme aus Taubenleber sind—wie Fraktionierungen mit Ammoniumsulfat zeigten—drei Enzymproteine beteiligt. Als Cofaktoren sind ATP, Coenzym A, DPN-H, TPN-H, Glutathion (oder Cystein) und Mn^{++} unerläßlich. Eine der Enzymfraktionen zeichnet sich durch einen bemerkenswert hohen Biotingehalt aus. Da die Fettsäuresynthese in vitro durch Avidin gehemmt und durch Biotinzusatz wieder in Gang gebracht werden kann, ist vermutlich Biotin bei dem Prozess beteiligt.

Die vorliegenden Befunde an den gereinigten Systemen zeigen, daß die Biosynthese der Fettsäuren keine genaue Umkehrung der β-Oxydation ist. Letztere ist ausschließlich in den Mitochondrien lokalisiert, erstere findet im Cytoplasma (vielleicht unter Beteiligung der Mikrosomen) statt. Vermutlich ist dies dadurch bedingt, daß in den Mitochondrien DPN-H bzw. TPN-H in nur geringen Konzentrationen vorhanden sind, da das in ihnen lokalisierte Elektronentransportsystem laufend DPN-H zu DPN oxydiert und der Quotient DPN/DPN-H infolgedessen weitestgehend zu Gunsten des DPN gelegen ist. Im Cytoplasma ist dies anders. Hier liegt der Quotient zu Gunsten von DPN-H bzw. TPN-H. Daher sind im Cytoplasma Hydrierungen und biosynthetische Prozesse begünstigt.

Der Umfang der Biosynthese von Fett im Organismus wird durch eine Reihe von endogenen und exogenen Faktoren beeinflußt. Diabetische Tiere zeigen eine

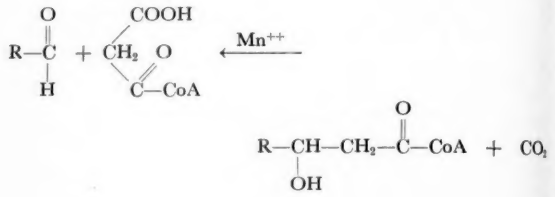
herabgesetzte Fähigkeit zur Fettsäuresynthese Umgekehrt wird diese durch Verabreichung von Insulin vergrößert. Auch die Nebennieren sind von Einfluß. Bei nebennierenlosen Tieren ist die Umwandlung von C^{14}-Acetat in Fettsäuren vermindert. Von einem großen Einfluß ist die Art der Ernährung. Im Hunger ist die Fettsäuresynthese in der Leber herabgesetzt. Nach den Untersuchungen von Catravas und Anker wird der Umfang der Fettsäuresynthese in der Leber durch einen bisher noch nicht identifizierten wasserlöslichen Faktor, der auch in der Hefe enthalten ist, reguliert. Im Hunger ist der Gehalt der Leber an diesem Faktor verkleinert. Daneben spielt aber auch wie im Kohlenhydratmangel oder beim Diabetes die Verminderung des Glucoseumsatzes im Pentosephosphatcyclus und damit eine unzureichende Bildung von TPN-H eine Rolle.

Bei der Biosynthese von Fettsäuren entstehen zunächst die Acyl-CoA-Verbindungen. Sie können den Synthesecyclus auf zweierlei Weise verlassen: *1*) Durch eine Deacylierung durch eine Deacylase. Diese hydrolytische Spaltung ist bei den Acyl-CoA-Verbindungen auf Grund ihres hohen Energiegehaltes durch die Thioesterbindung nicht reversibel. Sie bedeutet eine unökonomische Verschwendung von Energie. *2*) Durch eine Transferreaktion, in der die Acylreste von dem Coenzym A auf Glycerophosphat übertragen werden unter Bildung von Phosphatidsäuren. Hierbei wird die Energie der Acylmercaptanbindung unmittelbar zu einer synthetischen Leistung nutzbar gemacht. Die Phosphatidsäure wird dann durch eine Phosphatase zu einem Diglycerid dephosphoryliert. Das entstandene α,β-Diglycerid reagiert darauf mit einem weiteren Molekül Acyl-CoA unter Bildung eines Triglycerids.

Die Biosynthese von Phosphatiden geht ebenfalls von den α,β-Diglyceriden aus, Durch Reaktion desselben mit Cytosindiphosphatcholin entsteht Lecithin.

Das Enzym, das die Acylreste von dem Coenzym A auf das α-Glycerophosphat überträgt, hat ein stark ausgeprägtes Wirkungsoptimum für C_{16}-C_{18}-Fettsäuren. Dadurch ist die alte Erfahrung bedingt, daß man in den tierischen Fetten bevorzugt Palmitinsäure, Stearinsäure und Olsäure antrifft. Das Schicksal des bei der Fettsäurebiosynthese entstehende Acyl-CoA und die Stelle, an welcher es den Fettsäurecyclus verläßt, hängen also weitgehend von der Länge seiner C-Atomkette ab.

In der neueren Zeit wurde noch ein weiterer Mechanismus, durch den langkettige Fettsäuren gebildet werden können, entdeckt. Brady stellte fest, daß in der Taubenleber ein Enzymsystem vorhanden ist, das langkettige Fettsäuren aus Acetaldehyd und Malonyl-CoA in Gegenwart von TPN-H und Mn^{++} bildet. Hierbei findet eine Aldolkondensation zwischen dem Aldehyd und der aktiven Methylengruppe des Malonyl-CoA statt:



Gegenwart von ATP ist bei dieser Reaktion nicht nötig.

In analoger Weise entsteht Sphingosin durch eine Aldolkondensation zwischen Palmital und der aktiven Methylengruppe von Serin in Gegenwart von Pyridoxal-5-phosphat und Mn^{++}.

Hierbei wird zunächst Dihydrosphingosin gebildet, das dann durch ein Flavinenzym zu Sphingosin dehydriert wird.

Bei der Sphingosinsynthese durch Aldolkondensation bildet sich aus Pyridoxal, Serin und Mn^{++} ein Metallkomplex einer Schiffschen Base, durch den die Methylengruppe des Serins aktiviert wird.

Ein weiteres im Zusammenhange mit der Fettsäurebiosynthese zu behandelndes Problem ist die Verlängerung von Fettsäureketten um zwei bzw. mehrmals zwei C-Atome, die in den verschiedensten Versuchsanordnungen unter Verwendung von C^{14}-Acetat sowohl bei Versuchen in vivo als auch in vitro nachgewiesen worden ist. Stearinsäure und Fettsäuren mit einer noch größeren C-Atomzahl entstehen hauptsächlich von der Plamitinsäure ausgehend durch Kettenverlängerung. Nach den Befunden von Coniglio und Cate spielt die Kettenverlängerung insbesondere bei hungernden Tieren eine große Rolle. Sie kommt auch bei gut ernährten Tieren vor, bei diesen ist jedoch die Totalsynthese der höheren Fettsäuren aus C^{14}-Acetat bevorzugt.

Bei der Biosynthese von Fettsäuren entstehen primär gesättigte Fettsäuren. Diese können durch eine Dehydrogenase, die eine Doppelbindung zwischen den C-Atomen 9 und 10 einführt, zu den entsprechenden Monoensäuren dehydriert werden. Zumeist wird daher die Doppelbindung auch an dieser Stelle angetroffen. Mitunter werden auch Monoensäuren mit Doppelbindungen an anderer Stelle angetroffen. Über ihren Entstehungsmechanismus ist nichts bekannt.

Metabolism of bile acids

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THE EXISTENCE of a direct metabolic relationship between cholesterol and bile acid was conclusively shown by Bloch, Berg and Rittenberg (1943). After administration of randomly deuterated cholesterol to a dog with cholecystonephrostomy, they found that the cholic acid isolated from the urine was labeled to a highly significant degree. The finding of Bloch *et al.* was confirmed and extended in 1952. Fukushima and Gallagher (1952) studied the distribution of deuterium in the cholesterol molecule when labeled by exchange according to Bloch *et al.* They found that the deuterium was not uniformly distributed, about 50% being present in the terminal isopropyl group. Since this group was lost in the biological conversion of cholesterol to cholic acid they calculated that about 85% of the serum cholesterol had been converted to cholic acid in the experiment of Bloch *et al.* Several groups initiated investigations on the quantitative aspects of the conversion of cholesterol to bile acids when C^{14}-labeled cholesterol became available. Bergström (1952) found that 25% of intraperitoneally injected 4-C^{14}-cholesterol was excreted largely as taurocholate in the bile of a bile-fistula rat in 10 days.

The work of Chaikoff, Siperstein and coworkers (1952) and Siperstein and Chaikoff (1952) on the metabolic fate of C^{14}-cholesterol, labeled at C_4 of C_{26} with C^{14}, in the rat shed additional light on the qualitative as well as quantitative relationships in the transformation of cholesterol to bile acids. They found that 80–90% of the isotope of intravenously injected 4-C^{14}-cholesterol was excreted in feces in 15 days. When the isotope in the stools was fractionated, 90% was found present in the acid fraction and the rest in the neutral fraction. In rats with bile fistulas 30–40% of 4-C^{14}-cholesterol was excreted in the bile as bile acids in 2 days, while the feces from these animals contained only a few per cent of the isotope, mainly in the neutral fraction. No appreciable quantities could be recovered from urine or expired air. However, when intact and bile-fistula rats were given 26-C^{14}-cholesterol, the expired air contained $C^{14}O_2$ in about the same proportion of total dose as bile acids contained C^{14} after 4-C^{14}-cholesterol administration. The isotope recovered in bile and feces was mainly present in the neutral fraction. These results led these investigators to conclude that 80–90% of the cholesterol is ultimately metabolized to bile acids and the remainder is excreted as neutral steroids in feces. These conclusions concerning the major pathways of cholesterol degradation to bile acids have been demonstrated to be valid for other mammalian species, e.g. man and rabbit (Rosenfeld *et al.*, 1955; Siperstein and Murray, 1955; Ekdahl and Sjövall, 1955). A summary of the reactions by which the different bile acids of some species are formed is shown in figure 1.

The formation of deoxycholic acid and hyodeoxycholic acid through the action of intestinal microorganisms by the elimination of the 7α-hydroxy-group is illustrated in figure 2.

QUANTITATIVE ASPECTS OF BILE ACID METABOLISM

Quantitative studies of bile acid metabolism have recently been made mainly in the rat and human, and the results obtained in rats will be discussed first. The fact that the rat has no gall bladder emphasizes that caution should be exercised in assuming results obtained in this species as valid for other species.

Rat

Pool size and turnover. Studies of the distribution of labeled bile acids after intraperitoneal injection and equilibration have shown that the majority (about 70%) of the bile acids of the body are present in the small intestine. About 25% is found in the large intestine and only a few per cent in the liver and the bile ducts. The distribution in the intestines is possibly influenced by dietary factors. By using isotope dilution Eriksson (1959) has determined the pool of cholic acid in a 200 gm rat on a barley and oats diet. The small intestine, liver and bile ducts contained 10 mg and the large intestine 4 mg. With a somewhat different technique for determining pool size, Portman and Murphy (1958) have found a difference in the cholate pool with the type of diet: on Purina rat chow the amount of cholate was 9.9 mg/100 gm body weight in rats weighing approximately 200 gm, but only 4.5 mg on a synthetic diet with starch or sucrose as carbohydrate or with the addition of 20% indigestible material (Celluflour).

Lindstedt and Norman (1956, *a*) have found a half-life time of 2–3 days for cholic, chenodeoxycholic, lithocholic and cholanic acid (fig. 3). This value was arrived at by following the excretion of labeled acidic products in feces after intraperitoneal injection of a tracer dose of the 24-C^{14}-labeled acids. In a 200 gm rat

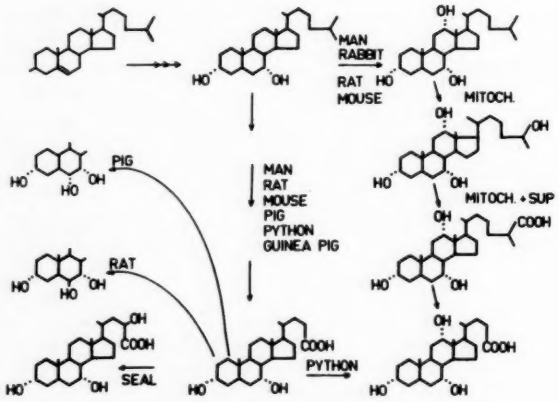

FIG. 1. Formation of bile acids in different species.

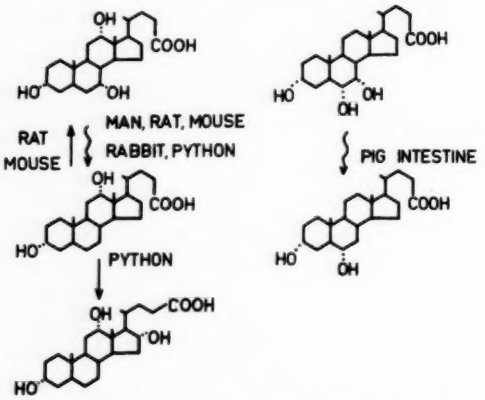

FIG. 2. Elimination of 7α hydroxyl by intestinal microorganisms.

with a pool size of about 14 mg of cholic acid, this corresponds to a production of 4.3 mg cholic acid per day. The total daily bile acid production can be obtained with this value by adding 0.7 mg representing the chenodeoxycholic acid production, i.e. 5 mg in a 200 gm rat.

In the rat, cholic acid which is secreted in the bile is partly converted into deoxycholic acid when it reaches the cecum. From this site it is incompletely absorbed and transported to the liver via the portal blood. The effective 7α-hydroxylating system, which is present in the liver, hydroxylates the deoxycholic acid to cholic acid and is thus responsible for the practically complete absence of the former acid in rat bile. The kinetics of these processes have been studied with 7β-tritium, 24-C^{14}-cholic acid. The tritium label in this acid is inverted in the formation of deoxycholic acid and lost in the subsequent 7α-hydroxylation in the liver. By following the ratio of tritium to C^{14} in the cholic acid pool, it has been found that about 50% of the total amount of cholic acid present in the body is converted to deoxycholic acid daily, the greater part of which is rehydroxylated to cholic acid in the liver (Lindstedt and Samuelsson, 1959). From analyses of portal blood from rats injected with labeled cholic acid, Olivecrona and Sjövall (1959) have arrived at similar results.

Homeostatic regulation of bile acid production. The physiological state of the bile acid production in the liver is seriously upset, when the enterohepatic circulation is broken by cannulation of the bile duct. This problem has been studied by determination of the amounts of cholic acid (Thompson and Vars, 1953, 1954) or of taurocholic and taurochenodeoxycholic acid (Erickson, 1957, *a*), that are excreted in the bile following cannulation of the bile duct. Most of the preformed bile acids present in the enterohepatic circulation are excreted during the first 12–24 hours, but then an increased synthesis is observed resulting in the formation of about 40–60 mg of bile acids, i.e. 10–20 times the normal synthesis. This occurs without any change in the blood cholesterol level.

Under these conditions the normal enterohepatic circulation is broken and no bile acids reach the liver. In recent experiments (Bergström and Danielsson, 1958) the bile acid production in bile duct cannulated rats was studied during continuous infusion of taurochenodeoxycholic acid into the lower part of the severed bile duct (fig. 3). This acid does not give rise to cholic acid (Bergström and Sjövall, 1954) thus permitting a determination of the excretion of cholic acid. The cholic acid synthesis was brought down into the range, found in the intact rat, when 5–10 mg per hour was injected into the intestine. These results indicate a homeostatic regulation of bile acid formation. The concentration of bile acids in the portal blood would be around 2 mg% according to these figures, and Olivecrona and Sjövall (1959) have established this value by chromatographic analysis of portal blood.

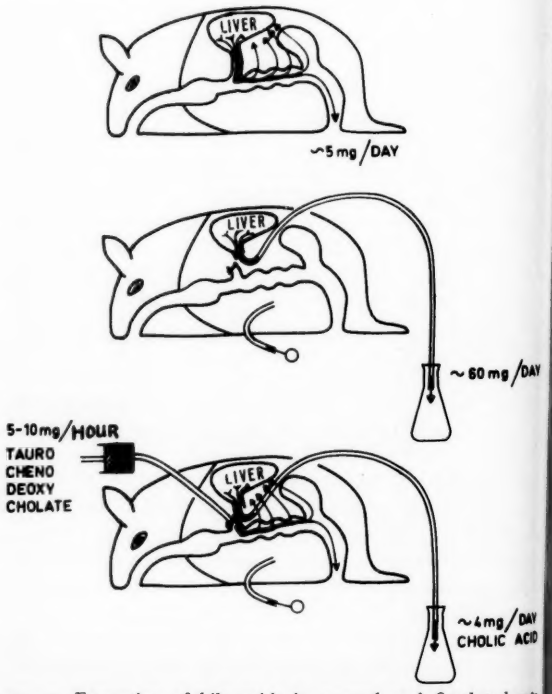

FIG. 3. Formation of bile acids in normal and fistulated rats.

Influence of thyroid hormone. Studies on the influence of hormones on bile acid formation and metabolism have been limited mainly to thyroxine. Byers and Friedman and collaborators (Rosenman *et al.* 1952, *a*, *b*, 1953) found an increased biliary excretion of cholesterol in hyperthyroid bile-fistula rats. This was confirmed by Thompson and Vars (1953, 1954) who in addition found a decrease in the excretion of cholic acid both in the hyper- and hypothyroid state. Also they were the first to observe that the cholic acid excretion of euthyroid bile fistula rats reached a plateau of about 35 mg per day about the third day after operation (fig. 4).

Eriksson (1957 *a*, *b*) in a detailed study of the biliary excretion of both cholic and chenodeoxycholic acid, in eu-, hyper- and hypothyroid bile-fistulated rats, found a decreased excretion of cholate in both hyper- and hypothyroidism. In addition, there was a very substantial increase in the amount of chenodeoxycholic acid in hyperthyroidism so that the total excretion of bile acids was at least the same as in normal (fig. 6). In hypothyroidism the amount of chenodeoxycholic acid excreted was less than in the euthyroid state. These results point to the possibility of a direct inhibitory effect of thyroxine on the 12α-hydroxylase, or a stimulatory one on the side-chain oxidation, as there is almost a reversal of the normal ratio cholic/chenodeoxycholic acid of 8:2 in the hyperthyroid state.

Influence of intestinal microorganisms. As mentioned earlier the half-life of the common bile acids has been determined to about 2–3 days in the normal rat. The profound effect of the microorganisms on bile acid turnover was first demonstrated in rats treated with 'intestinal antibiotics.' In these animals a half-life for cholic acid of about 10 days was found (fig. 5) (Lindstedt and Norman, 1956 *b*).

This problem has been further studied by Bergström and coworkers in collaboration with B. Gustafsson using his colony of germ-free rats reared at Lund (Gustafsson *et al.*, 1957). In figure 6 is shown the normal semilogarithmic plot of the excretion of cholic acid in a rat under complete germ-free conditions as well as after infection with *Aspergillus niger* and *Clostridium perfringens*.

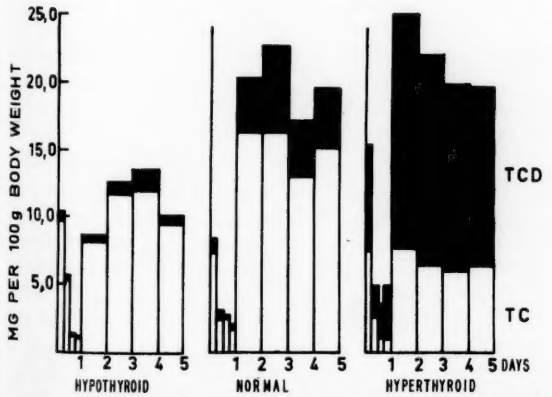

FIG. 4. Excretion of bile acids in hypothyroid, euthyroid and hyperthyroid bile fistula rats. TC = taurocholate; TCD = taurochenodeoxycholate.

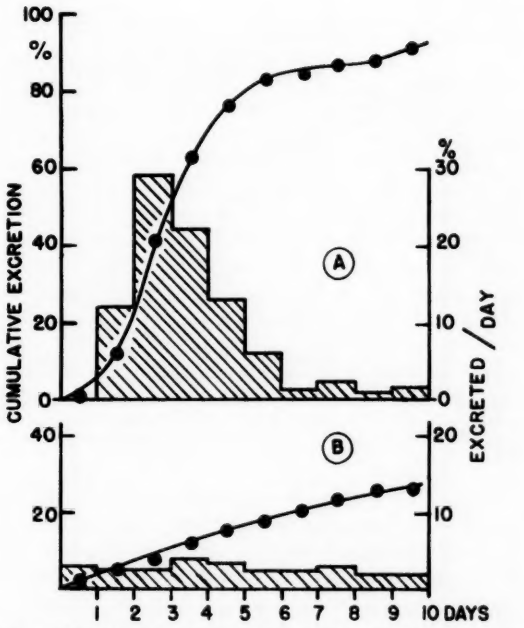

FIG. 5. Excretion of isotope in feces after intraperitoneal injection of 24-C^{14}-cholic acid into a normal rat (A) and one treated with chemotherapeutics (B).

As can be seen there is no change in the rate of excretion under these conditions ($t_{1/2}$ = 10 days); however, after total infection an immediate return to the normal half-life is found ($t_{1/2}$ = 2.5 days). In recent experiments on normal, *E. coli*-infected and germ-free rats, essentially the same amount of bile acids present in the body was found in the different groups, whereas the half-life time of cholic acid was about 2–3 times longer in the two last-mentioned groups (Gustafsson, Norman and Sjövall, 1959).

These results show that there is a pronounced decrease in the synthesis and excretion of bile acids in animals where the appropriate microorganisms are lacking. The intestinal flora has thus a profound influence and possible changes in it must also be considered when dietary changes are made.

Influence of diet. The effect of various types of diets on the bile acid metabolism has been studied by Portman, Mann and coworkers (Portman and Mann, 1955; Portman, Mann and Wysocki, 1955). Their earlier findings that the bile acid excretion in bile-fistula rats is considerably smaller, with a purely synthetic diet ininstead of ordinary laboratory diet (Purina rat chow), and that the use of sucrose instead of starch in the synthetic diet decreases the excretion even more, have been confirmed and extended in a recent study (Portman and Murphy, 1958) using a different experimental approach.

In this connection the profound influence of the intestinal flora on the qualitative as well as quantitative pattern of the bile acid metabolism must be borne in mind. Thus, changes of the bile acid excretion on different diets might be due to changes in the intestinal flora brought about through drastic changes of the diet.

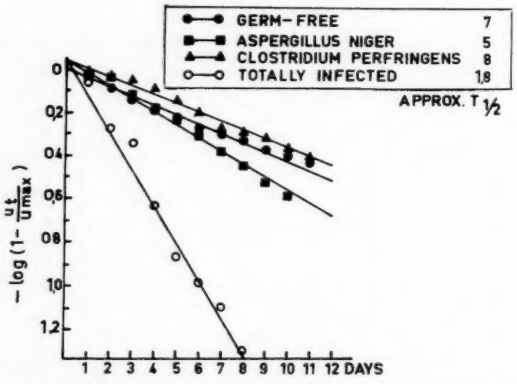

FIG. 6. Elimination of 24-C^{14}-cholic acid by a rat during periods with different conditions in the intestine (semilogarithmic plot).

When studying the bile acid production in fistulated animals, it must also be observed that the excretion during the first 24 hours consists of the circulating pool of bile acids, and that the normal synthesis of bile acids in liver progressively increases during the first few days after fistulation before reaching a plateau, that in the rat might be 10 times the normal rate of synthesis. The response of the bile acid production to different diets at this time thus represents the variations of the maximal capacity of the liver to produce bile acids when no bile acids are coming to the liver via the portal blood. However, both the pool size and the synthesis in intact animals might vary independently of this parameter.

Man

In human bile the main bile acids are cholic, chenodeoxycholic and deoxycholic acid, conjugated with glycine and taurine. Deoxycholic acid has been shown to be a bacterial metabolite of cholic acid (Lindstedt, 1957 *d*). The proportions among different bile acids in hydrolyzed bile has been determined by Wootton and Wiggins (1953) and Isaksson (1954). The former authors have also reported the analyses of the conjugated bile acids in 3 human bile samples (Wiggins and Wootton, 1958). Using quantitative paper chromatography Sjövall (1959 *c*, *d*) has recently made a survey of the quantitative relationships between the main bile acids in bile and intestinal contents from healthy and diseased humans. The ratio between glycocholic, glycochenodeoxycholic and glycodeoxycholic acids in gall-bladder bile was 1.1/1/0.6, values in agreement with those reported by Isaksson for the free acids. The ratio between glycine and taurine conjugates was 3.2. It was also found that cholic, chenodeoxycholic and deoxycholic acid are conjugated in the same proportion with glycine and taurine. The variations in the glycine to taurine ratio were, however, considerable. Also in the same person this ratio varied in samples of duodenal contents collected on different days.

The proportion between the different acids stayed fairly constant during and after absorption. The concentration of bile acids immediately after the gall bladder emptied, was 13–46 mEq/l. and 2.5–10 mEq/l. during the period of digestion and absorption. During fasting the concentration was 5–10 mEq/l. In a cholecystectomized person the value was of the same order of magnitude.

Analyses of gall-bladder bile from diseased persons showed that there was a decrease in the normal ratio glycine/taurine in several cases of liver disease, while there was a considerable increase in 2 cases of hypothyroidism. Deoxycholic acid was absent or present in much smaller amounts than normal in case of long-standing obstructive jaundice, acute hepatitis and portal cirrhosis. The amount of cholic acid was low in portal cirrhosis, indicating an impairment of the 12α-hydroxylation in this disease.

Pool size and turnover of bile acids in man. The pool and turnover of cholic acid in healthy humans has been determined by Lindstedt (1957 *c*). The approach to this problem has been to administer a tracer-dose of 24-C^{14}-cholic acid and follow the decrease of the specific activity of the cholic acid in bile. The cholic acid was isolated from small samples of bile collected by duodenal intubation, after intravenous injection of cholecystokinin. The pool of cholic acid was found to be 1.4 gm with a half-life of 2.3 days. On the basis of the ratio between cholic, chenodeoxycholic and deoxycholic acid, determined by quantitative paper chromatography, the amount of chenodeoxycholic acid was calculated to be 1.45 gm and of deoxycholic acid to 0.77 gm, giving a total bile acid pool in man of 3.6 gm. However, as deoxycholic acid is formed from cholic acid, only the sum of the latter and chenodeoxycholic acid is used in the calculation of the total formation of bile acids.

On the basis of these figures for half-life and pool of cholic and chenodeoxycholic acids the amount of cholesterol degraded to bile acids daily in these cases can be estimated to about 0.7 gm. A summary of the turnover of cholesterol in man is given in fig. 7.

Ahrens and Lindstedt (1959) have recently studied the turnover of cholic acid in 2 patients with essential hypercholesterolemia. During the experimental period the patients were fed a formula diet, rich in unsaturated fatty acids, and their serum cholesterol level had been forced down. Under these conditions, the half-life for cholic acid in these patients was about 3 times longer than in healthy persons on uncontrolled diet and the cholic acid pool considerably smaller.

Influence of diet. The demonstration by Siperstein and Murray (1955) and Lindstedt (1957 *c*) that the bile acid formation is a quantitatively very important parameter of the cholesterol metabolism in man has stimulated studies of the variation of the bile acid metabolism in relation to diet in normal subjects and in different pathological conditions in a number of laboratories. The scanty and partly contradictory data available so far hardly merit a review, but the work published so far will be briefly mentioned.

Gordon, Lewis, Eales and Brock (1957 *a*, *b*) report that the 'total fecal bile acids' increased from 128–158

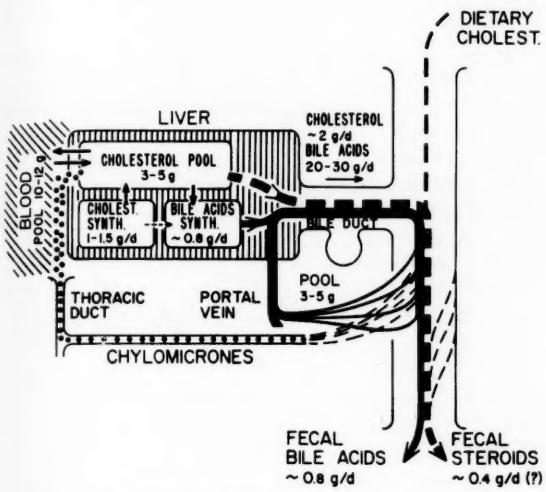

FIG. 7. Summary of turnover of cholesterol in man.

mg/day on a low fat or hydrogenated coconut fat diet to 258–338 mg/day on a sunflower seed diet, that caused a reduction of the plasma cholesterol level. Haust and Beveridge (1958) with a similar analytical method have reported comparable data.

Lewis (1958) reported also a rise in bile acid excretion in bile fistula patients with highly unsaturated fats, i.e. a reversed relation between the variations of plasma cholesterol and 'total fecal bile acids'.

On the other hand, Hellman, Rosenfeld, Insull and Ahrens (1957, 1959) reported, in relation to changes in the dietary fat and plasma cholesterol, no significant change in the fecal acidic metabolites of C_4-labeled cholesterol, while they noted large changes of the excretion of neutral sterols of a magnitude that reciprocated the changes in the plasma cholesterol.

It must be pointed out in this connection that drastic changes in the fat of the diet might cause large changes in the intestinal flora and that the methods used for analysis of the total bile acids in the feces apparently account for only part of the bile acids, since often a very large part is transformed by microorganisms into products that are not determined with the methods used.

Formation and metabolism of conjugated bile acids. Bile acids occur in the bile conjugated with taurine, and in mammals also with glycine. In certain lower animals (amphibiae, sharks, etc.) sulphates of steroid alcohols apparently fulfill the same function as the conjugated bile acids (Haslewood and Wootton, 1950; Haslewood, 1955).

The mechanism of conjugation of the bile acids and the metabolism of the peptide bond in the conjugated bile acids have been elucidated to a large extent. Siperstein (1955) as well as Bremer and Gloor (Bremer 1955 *a*, *b*, Bremer and Gloor, 1955) have shown that in rat liver the conjugation of cholic acid with taurine and glycine is effected by microsomes with the addition of DPN, ATP, Mg^{++}, nicotinamide and CoA. Bremer (1955), Elliott (1956), and Siperstein and Murray (1956) have independently obtained evidence for the formation of cholyl-CoA as an intermediate.

The conjugation of cholic acid in human liver homogenates both in normal persons and in relation to various diseases has been studied by Bergström and Gloor (1954 *a*) and extensive work has been done by Ekdahl (1957, 1958) employing a micro-method developed by Ekdahl and Gloor (1957), which requires only 10–30 mg of liver tissue, obtained for instance by percutaneous liver puncture. Liver homogenates from healthy persons were found to conjugate cholic acid with glycine and taurine in a ratio of 1.2:1. In a study of the conjugation of cholic acid in liver homogenates from 150 cases of different liver diseases, carcinoma of pancreas or stomach, peptic ulcer, etc., Ekdahl (1957, 1958) found that there existed a correlation between the type of disease and the pattern of bile acid conjugation. The common denominator seemed to be a decrease in the ratio of glycine to taurine conjugation, when the liver was diseased.

Bergström and Gloor (1954) observed that a 3-fold excess of taurine over bile acid in the human liver homogenate changed the pattern of conjugation to a 90 % conjugation with taurine, while an addition of excess glycine had no effect. A similar response of healthy humans to an excess of these amino acids has been shown by Sjövall (1959 *b*), who found that feeding increasing amounts of taurine up to 1.5 gm per day increased taurine conjugation, while 21 gm of glycine daily had no effect.

However, an increase in glycine conjugation has been shown in cystine-deficient rats by Eriksson (unpublished) and also in B_6-deficiency (Bergeret and Chatagner, 1956), in contrast to the almost exclusive taurine conjugation normally present.

The ratio between glycine and taurine conjugates in bile and duodenal contents has been determined by Sjövall (1959 *c*, *d*), and Encrantz and Sjövall (1959) in newborn babies and children, and in healthy and diseased adults.

In the first days after birth the bile acids are conjugated mainly with taurine, but during the first week the ratio of glycine to taurine conjugates increases rapidly reaching 'adult' values after the first years of life. The mean ratio in healthy subjects was 3.2:1, fairly large variations being found at different times in the same individual (possibly due to dietary influence). In liver disease, especially in long-standing obstructive jaundice, there is a decrease in this ratio, results that agree with those found by Ekdahl for the conjugation of cholic acid *in vitro*.

After operative release of the obstruction the glycine: taurine ratio rapidly rises to values often higher than the normal (Ekdahl and Sjövall, 1957).

The metabolism of the peptide bond of the conjugated bile acids during their enterohepatic circulation and passage through the intestinal tract has been studied in rats mainly by Portman and coworkers, and Bergström, Gustafsson, Lindstedt and Norman. Portman and Mann (1955) found that most of orally administered S^{35}-taurocholate was absorbed without hydrolysis, as about

70% of the isotope was recovered in the bile of bile-fistula rats. Norman (1955 *a*) has shown that glycine-conjugated cholic acid, which is normally found only in small quantities in rat bile (1–2%), is excreted unchanged when injected intraperitoneally into bile-fistula rats. This indicates that the liver does not attack the peptide bond, and this contention has been strengthened by the finding of Lindstedt and Norman (1957) that intraperitoneally administered taurocoprostanic acid is excreted unchanged in bile of bile-fistula rats, while free coprostanic acid (Bridgwater and Lindstedt, 1957) under the same conditions is excreted mainly as taurocholic acid. During the enterohepatic circulation of the bile acids there is a considerable hydrolysis of the peptide bond caused by intestinal microorganisms and a conjugation in the liver, as demonstrated by the studies of Norman (1955 *a*) on the metabolism of perorally administered glycine conjugated 24-C^{14}-cholic acid. This compound was rapidly absorbed, but after 6 hours of enterohepatic circulation 50% of the 24-C^{14}-cholic acid was present in bile as taurocholic acid.

In the intestinal lumen the conjugates are split by the action of bacteria and normally only free bile acids are found in feces. The observation that germ-free reared rats (Gustafsson *et al.*, 1957) or rats treated with antibiotics (Norman, 1955) excrete only conjugated bile acids in feces demonstrates that the intestinal microorganisms are solely responsible for this reaction and thus that the extent of hydrolysis caused by intestinal proteolytic enzymes must be very small.

The microbial splitting of the conjugated bile acids does not start until these have reached the cecum (Norman and Sjövall, 1958). This reaction has been studied *in vitro* by Norman and Grubb (1955) who found that of normal inhabitants of the intestinal tract several species of *Clostridia* and enterococci were capable of hydrolyzing the peptide bond, while *E. coli*, *Proteus*, etc., were not.

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Lipids and atherosclerosis

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THE WIDESPREAD OPTIMISM that the problem of atherosclerosis is not insoluble, is signified by the enormous volume of literature that has accumulated during the last decade. It is quite impossible, in a short space, to review completely the different approaches to this problem. Instead, in an international congress one may be allowed the prerogative to illustrate how the approach in one's own area is influenced by research elsewhere.

There is much evidence to suggest that atherosclerosis is the algebraic product of time and a disordered fat metabolism. There is much evidence too, to suggest that this disordered fat metabolism arises from dietary causes. The hypothesis in its simplest form suggests that an imbalanced diet with regard to fats or some factor closely associated with dietary fats, leads over a course of time to a disordered fat metabolism resulting in atherosclerosis which is expressed clinically by certain effects—perhaps most strikingly as ischemic heart disease. This hypothesis can explain both the epidemiological and biochemical features of the disease but, despite this, universal acceptance is not at hand. It behooves us therefore to reexamine constantly and critically the facts, old and new, that form the strength of the links in this triple association, otherwise there will be no progress towards the solution of this, the major cause of death in the western civilized countries.

Few will deny that an environmental factor is at play even though it is not easy to differentiate those factors contributed by heredity from those occurring in the environment. Undoubtedly the striking differences of opinion on the particular factor or factors responsible in our present day mode of life arise from different outlooks on certain fundamental aspects of the disease. In this triple association there is strong evidence for a direct relationship between the diet and serum cholesterol trends in man, but the strength of the other links depend on certain assumptions which, as yet, have not been established as fact. It has yet to be shown that these dietary effects on the serum lipid levels have any bearing on the degree of atherosclerosis, and secondly whether or not there is justification for the assumption that atherosclerosis and ischemic heart disease bear a cause and effect relationship. There certainly seems to be no justification for the assumption that the severity of aortic atherosclerosis parallels that in the coronary vessels (29, 31).

RELATIONSHIP OF CORONARY ATHEROSCLEROSIS TO ISCHEMIC HEART DISEASE

Ischemic heart disease was defined (89) as a cardiac disability, acute or chronic, arising from reduction or arrest of blood supply to the myocardium in association with disease processes in the coronary arterial system. It is generally accepted that atherosclerosis forms the basis of this occlusive disease but its relationship to myocardial infarction is not clear. Despite the rapid rise in mortality from ischemic heart disease over the last few decades, Morris (68) has claimed that prevalence of severe atherosclerosis has declined. In animals atherosclerosis is easily produced, but until recently the classical picture of myocardial infarction has not been produced with any regularity by a similar means. The reported sex difference in the prevalence of severe atherosclerosis found at autopsy (54) is far less than the remarkable sex difference in the incidence of myocardial infarction (73). It is well known, too, that severe occlusive disease of the coronary arteries may exist without apparent ill effects, but the problem relating the severity of coronary atherosclerosis to its effects is made even more difficult when one notes that in a large proportion of cases with myocardial infarction at autopsy no subtending occlusion was found despite careful dissection (15, 39, 61, 91). Morris's use of calcification and other criteria for the index of severity or acuteness of the pathological process may be open to question. Atheroma, as a term, may cover a wide variety of lesions each differing in its significance. Montgomery (66), apart from emphasizing the importance of the blood supply through the coronary arterial system as a whole, draws attention to the distinct differences of the 2 extremes, soft atheroma and hard atheroma, and all the varieties between these 2 extremes, and suggests that it is possible that these arise by different mechanisms.

It is conceivable, therefore, that in addition to atherosclerosis a process may exist for the full explanation of the clinical manifestations. This has revived a great deal of interest in thrombotic and other mechanisms that interfere with circulatory flow, whereas the particular anatomical structure and the mechanisms regulating blood flow in the coronary circulation would give rise to different effects from atheroma in other circulatory systems. Ischemic heart disease then may be

the end result of a series of processes, not least in importance being the state of the collateral circulation and the functional status of the heart at the time.

PATHOGENESIS

The essential components of the human atherosclerotic plaque appear to be intimal fibrosis and lipid infiltration, and the predominance of one over the other may determine the hardness or softness of the plaque. It is therefore not unnatural that most theories on the pathogenesis centre around either fat, fibrin or both, but considerable controversy exists as to which component is primary and which is secondary. The filtration theory (74), the intimal hemorrhage theory (75), and the thrombogenic theory revived by Duguid (27) can account for the focal distribution of atherosclerosis at sites of pressure or velocity change within the circulatory system. The major difficulty in the acceptance of the latter 2 theories is the explanation for the fat present in the plaque. The extent to which this can occur does not encourage one to accept the view that the fat present is the product of blood destruction from small hemorrhages recurring over long periods (28). The filtration theory too could more easily explain the fatty streaks, but recently fluorescent antibody techniques have shown that a thin film of fibrin covers them (23). Astrup (7) has suggested that atheroma represents a disturbance in the balance of fibrin deposition and fibrin removal, and recently a great deal of interest has centered around fibrinolysis as the possible pathogenetic mechanism. Cholesterol and its esters are in high concentration in the lipid of the atheromatous plaque and current *in vitro* studies illustrate the great affinity of fibrin for cholesterol, and certain of its esters. (Table 1, fig. 1.) Little affinity appears to exist between fibrin and triglyceride and phospholipids in these *in vitro* studies. Cholesterol oleate, furthermore, inhibits fibrinolysis (64), so that it is possible that the lipid enters the atheromatous plaque at the time of fibrin formation and a large amount of lipid within the plaque may represent its earliest phase.

TABLE 1. *Optical Density Units*

	Clotting		% entry into Clot
	Before	After	
Lipemic plasma	143	139	3
Fasting plasma plus	17	17	0
Cholesterol	140	77	45
Cholesterol acetate	112	32	71
Cholesterol stearate	210	25	88
Cholesterol oleate	860	37	96

Optical densities (×1000) of 1 in 10 diluted plasma before clotting with thrombin and the retracted fluids after removal of the clot.

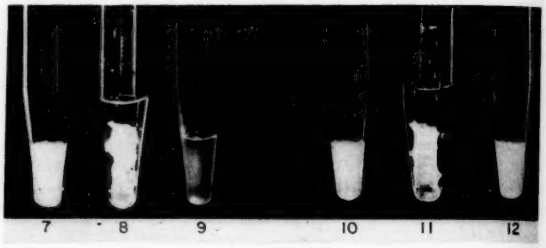

FIG. 1. Effect of clotting a diluted fasting plasma solution containing cholesterol oleate (tubes 7–9) and lipemic plasma (tubes 10–12). Tubes show successively the solutions prior to clotting, the clot adherent to the glass rods placed in buffered saline and retracted fluids. As clot lysis commences the cholesterol oleate begins to opacify the buffered saline (see table 1).

RELATIONSHIP OF SERUM LIPIDS TO ATHEROSCLEROSIS AND ISCHEMIC HEART DISEASE

Lipids in the Atheromatous Plaque

Earlier studies (87) drew attention to the fact that the lipid of the atheromatous plaque resembled that in the plasma. The arterial wall, however, is capable of synthesising cholesterol (22). Unlike the plasma there is more free cholesterol than esterified cholesterol in the plaque and a higher cholesterol phospholipid ratio than that present in plasma (55). Plaques are said to contain carotenoid pigment (13) which cannot be synthesised and migrates electrophoretically with the beta lipoproteins. This evidence suggest, therefore, that at least some of the fat present is deposited there.

Our studies on the component fatty acids of the esters in the plaque, together with those reported from elsewhere (14, 55, 85, 90), do not support the view that the presence of the more saturated cholesterol esters favours deposition as the proportion of saturated acids is lower than in the plasma. In this way, they resemble more the component acids of the β lipoprotein than the plasma as a whole. Widely divergent findings however are seen in these few recent studies, and with the more widespread use of present day sensitive techniques these discrepancies may be clarified. On the other hand they may merely represent the dynamic changes occurring within the plaque or the different stages in the pathology.

Serum Lipids in Ischemic Heart Disease

For nearly half a century (5) the very close relationship that exists in the experimental animal between changes in the serum lipids and the development of atherosclerosis has been universally accepted. Atherosclerosis could be expressed as an algebraic product of the height of the serum cholesterol and the duration of its elevation. Unfortunately these animal experiments have been mostly artificial in that serum lipid levels have been extremely high and duration of experiment very short. It is for these reasons probably that the resultant lesions, in most instances, bears little resemblance to the full-blown picture in man.

It is well accepted too that the incidence of ischemic heart disease and the prevalence of severe atherosclerosis is greater than expected in known disorders of lipid

metabolism such as essential xanthomatosis, diabetes mellitus and myxoedema. It is of paramount importance, therefore, to determine firstly if a disorder in fat metabolism really does exist in ischemic heart disease and secondly to define precisely the nature of the disorder and study its mechanism.

Cholesterol lipoprotein system. The most publicized metabolic difference between patients and controls concerns the cholesterol lipoprotein complex in the serum Amongst the many factors that constitute this lipoprotein complex, most emphasis has been placed on the more easily estimated serum cholesterol. Certainly where wide differences in susceptibility to ischemic heart disease exist, there are wide parallel differences in mean serum cholesterol levels. In studies on more homogeneous groups, which applies particularly to clinical practice, there is considerable overlap between patients and controls (17).

A great many attempts therefore are being made to define more precisely the metabolic disorder that exists in ischemic heart disease. Being insoluble in plasma and other watery media in the body, fats have to be combined with cholesterol, phospholipids and proteins in the form of lipoproteins which are readily soluble. From this has arisen the use of various lipid indices. There is the atherogenic index (33), but unfortunately this necessitates the use of the ultracentrifuge. Phospholipids are said to be stabilizers of cholesterol solutions (3, 83) and from this has arisen the concept of the cholesterol phospholipid (C/P) ratio which has been shown to be higher in the patients than in the controls (32). Again due to considerable overlap, particularly in the older age groups (71), the serum C/P ratio has fallen largely out of favour.

Electrophoretically, 2 major lipoprotein classes occur, the alpha or faster migrating component and the beta. The serum electrophoretic lipoprotein pattern is different in different species. As atherosclerosis develops in the experimental animal the pattern changes, and in man it is influenced by both age and sex. Age, masculinity and ischemic heart disease favour a higher proportion of cholesterol in the beta fraction (9, 70). In epidemiological studies greater differences are seen when the serum is fractionated in this manner. In Cape Town we have been impressed by these differences and with the newer techniques of heparin precipitation and gas-liquid chromatography. Dr. Fewster has studied recently the composition of the beta lipoproteins in fasting age-matched Bantu and White males in the fifth decade, the latter with and without ischemic heart disease.

Despite differences in means of the order of 84 mg % in the total serum and 100 mg % in the beta cholesterol levels between the 2 extremes, namely the Bantu and the patients with ischemic heart disease, the pattern of the fatty acids esterified with cholesterol in both the total serum and the beta fraction was almost identical. In both groups the beta cholesterol esters were quite different from those in the unfractionated serum. Contrary to expectations there was a significantly greater amount of linoleate and less of saturated acids in the beta cholesterol ester. On the other hand, there was a striking similarity between the fatty acid pattern of the glycerol esters in the beta and unfractionated serum in both the Bantu and the White controls. In the patients, however, there were differences with the beta containing significantly more saturated acids and less oleate than in the unfractionated serum.

Serum triglycerides. Recently, interest has been focussed on quantitative differences in the serum triglycerides. In fat tolerance studies it had been noticed that the opalescence of the serum in the fasting state was greater in the patients than in the controls (10, 19, 65). In Cape Town, counts on I^{131} labelled triolein studies, carried through to the following day, have shown a higher activity in the patients than in the controls. Albrink and Man (4) have suggested that an error in the metabolism of triglycerides is the lipid abnormality operative in ischemic heart disease as less overlap between patients and controls was found when differentiation was made on the triglyceride levels than on serum cholesterol levels. They place their upper level at 160 mg %. Antonis and Bersohn (personal communication) from their study on the effect of age on the serum triglyceride levels in the Bantu and White subjects place their upper level at 114 mg %. Their mean level for the patients with ischemic heart disease was found to be 196 mg %.

Fat tolerance tests. The lipemia following a standard fat meal is more intense and more prolonged in patients with ischemic heart disease than in age matched controls when compared on a group basis (10, 19, 65). Usually, considerable overlap between patient and control groups occurs and it is not until late in the lipemic phase that the differences become statistically significant. Due to this it was considered that the patients lacked a sufficiency of the clearing factor and certain experiments using heparin to activate clearing substantiated this claim (72). *In vitro* studies failed to confirm this view (65). The heparin activated clearing mechanism in patients and controls behaved identically when the enzyme was incubated with substrate, whether this be lipemic plasma from patient or control. Neither in the enzyme nor the substrate could the explanation for the different lipemic responses of patient and control be found, but there may be little in common between the heparin activated clearing mechanism and that occurring naturally.

As the degree of lipemia at any stage represents the algebraic sum of absorption and clearing, the same fat, as fed orally, was administered intravenously to patients and controls in Cape Town by Bouchier (unpublished observations). Differences were seen after oral feeding but not following intravenous administration. These studies suggest then that the differences in the lipemic response in fat tolerance studies between patients and controls probably reside in absorptive mechanisms. Why this is so has yet to be determined.

Metabolic Effects During Lipemic Phase

The bearing these lipemic differences have on pathogenetic mechanisms of atherosclerosis and ischemic heart disease is unknown. Prolongation of the lipemic phase would allow prolonged contact of the lipid with the arterial intima and possibly subsequent deposition and qualitative differences in lipid structure, chemically or physically, could govern individual susceptibility (67). Chemically, however, there appears to be a tendency to keep the pattern of serum fatty acids constant during the lipemic phase (26, 76), but this needs further study.

Prolongation of the lipemic phase could give rise to other mechanisms. Exacerbation of anginal pain, with ballisto- and electrocardiographic changes have been noted during the lipemic phase (51), but these findings remain unconfirmed despite the many fat tolerance tests performed since. A circulatory overload of fat is poorly tolerated by certain animals. This has been attributed to widespread tissue ischemia due to intravascular obstructions from fat globules and aggregated red cells (81, 82). Possible effects of plasma fat on red blood cells could account for the decrease in electrophoretic rates of erythrocytes with age and in patients with atherosclerosis (24). Washing the erythrocytes and suspending them in saline abolished these differences, while treating them with different fatty acids revealed that the surface activity was proportional to the degree of unsaturation.

The effect of lipemia on blood coagulative mechanisms remains a controversial subject. All are agreed that the plasma stypven time is shortened during the lipemic phase and that ethanolamine phosphatide plays an important role in this regard (77). Other methods of assessing fibrin formation or fibrin removal do not appear to give consistent results (62). Like Hougie and Ayers (43), we have been unable to confirm the finding that fibrinolytic activity is depressed following a fat meal (21, 37). We were unable too to exhibit differences between patients with ischemic heart disease and their age matched controls. On the other hand, continuation of these studies in Oxford revealed that when various fats were added *in vitro* to the system, only cholesterol oleate inhibited fibrinolysis significantly (64). Large differences in serum cholesterol concentration exist between Bantu and South African White, and the mean clot lysis time of the Bantu is nearly half that of the White (62). In rabbits, clots in the ear veins lysed more slowly when a high cholesterol diet was fed (52), but otherwise no correlation between lysis times and serum cholesterol levels has emerged (43). Possibly these inconsistencies are due to the insufficiently refined methods used.

One may conclude that from the chemical analysis of the atheromatous plaques, and when comparisons are made on groups with wide differences in susceptibility to ischemic heart disease, the findings could suggest that the disease results from a disorder in lipid metabolism. In more homogeneous groups, particularly at the age at which the disease is most common, considerable overlap is seen. As yet the serum lipid abnormalities at present recognized in ischemic heart disease cannot be used with confidence to predict its development in the individual. In studies on more homogeneous groups the overlap may represent merely a graded characteristic or outline the difficulty of defining controls amongst whom there may be some in the process of transferring to the patient group. The apparent tendency to maintain the plasma fatty acids constant might encourage more profitable excursions into studies of body lipids other than those in the blood, but these relatively minor differences may merely emphasize the importance of other factors in the development of ischemic heart disease in its final form. Nevertheless, the differences are sufficient to stimulate further study as techniques become more refined, and few will deny the advantages that would accrue should one have at hand a characteristic whereby the early stages of the disease can be recognized.

DIETARY LIPIDS AND SERUM LIPIDS

Again when comparisons are made between groups with wide differences in susceptibility to ischemic heart disease, there are wide differences in the fat content of the diets. These differences are both quantitative and qualitative (20). Earlier in the decade, claims were made to different effects of animal and vegetable (seed) dietary fats on the serum cholesterol levels (38, 40, 50). Confirmation of these differences was soon forthcoming from many centers, but these controlled feeding studies showed that the difference was not animal versus vegetable but was due to differences in the fatty acid composition (1, 12, 18, 58). The serum cholesterol level is raised by fats and oils containing the more saturated fatty acids whereas it is lowered by those with a high content of the polyunsaturated fatty acids, and these effects are seen as long as the fat is fed whether it be over a few days or several months (6, 35).

The mechanism for such effects is less well understood. The sharp increase in bile acid excretion on feeding the high iodine value oils (53) has implied that turnover rates are accelerated. The effect of hydrogenation of an oil on its serum cholesterol response led one at first to believe that the essential fatty acid content was the deciding factor; particularly seeing that these fatty acids possess biological activity other than the supply of calories. The hypothesis was attractive too, in that in the experimental animal they appeared to be necessary for the metabolism of the more saturated fatty acids and the requirement was proportional to the total fat intake (42). Seal oil, fish body oil and cod liver oil depress serum cholesterol levels, but menhaden oil is unable to cure essential fatty acid deficiency in the rat (2). Fatty acids essential to the rat need not be identical with those in man.

Our recent experiments on egg fractionation have focussed our attention back to the role of exogenous and

TABLE 2. *Mean Change in Serum Cholesterol Levels During 10-day Feeding Periods, Following an Isocaloric and Isonitrogenous Fat-free Diet*

Domestic fowl	+47	Duck	+40
Ostrich	+46	Penguin	+24
Acetone insoluble fraction			
(a) Protein			−5
(b) Phospholipid			+6
Acetone soluble fraction			
(a) Mother liquor			+46
(b) Unsaponifiable fraction			+5
(c) Saponifiable fraction			+10
(d) Unsap. + Sap. mixed			+41
(e) Unsat. + Sap. unmixed fed together			+40
(f) Unsap. + Sap. unmixed fed 6 hours apart			+15

endogenous cholesterol. Under our experimental conditions, egg yolk lipid from the domestic fowl elevates the serum cholesterol levels higher and more rapidly than any other fat on a gram for gram basis. It has, however, a relatively high iodine value (I.V.70) and the linoleic acid content varies from 16% to 19%. Ostrich's egg and duck's egg lipid have a similar composition and produce almost identical serum cholesterol raising effects. Penguin egg lipid (I.V.80) however produces much less of a rise in serum cholesterol levels (table 2). Removal of the phospholipid and protein content of egg yolk does not interfere with the rate and extent to which the serum cholesterol rises on feeding the acetone soluble fraction. If this fraction is then saponified, neither the saponifiable fraction nor the unsaponifiable fraction elevate the serum cholesterol levels to any significant degree if they are fed separately. If they are mixed, either by redissolving in the laboratory or fed together off the same plate at the same time, elevation of the serum cholesterol levels occurs. If, however, one fraction (say, the unsaponifiable) is fed and an interval of 6 hours is allowed to elapse before feeding the other, no marked rise in serum cholesterol levels follow. Similar effects are seen if pure cholesterol is used instead of the unsaponifiable fraction and if a fat of similar iodine value viz. hydrogenated sunflower seed oil is used instead of the saponifiable fraction. It would appear then that for a serum cholesterol raising effect both the sterol content and the fatty acid composition of a fat is important.

On the other hand a potent serum cholesterol elevator is hydrogenated coconut oil which is free of cholesterol. Wilkens (88), from our unit, believes this can be explained on the simple principle of the solubility of cholesterol within an oil or fat. His *in vitro* studies show that cholesterol is more soluble in the more saturated fats and oils and less soluble in the highly unsaturated oils. His range of solubility percentages (fig. 2) exactly parallel the serum cholesterol raising or lowering effects of the fats or oils tested. The serum cholesterol raising effect of an oil such as coconut oil, which is free of cholesterol, is explained by its high solubility for endogenous cholesterol. Sitosterol competes for the solubility. It is possible but less easy to explain the serum cholesterol lowering ability of intravenous cottonseed oil in this manner (36), but this still does not vitiate the hypothesis that the response of the serum cholesterol to a particular fat or oil could be determined at gut level.

Other Dietary Factors

Undoubtedly one must take account of other dietary factors but we have seen no convincing effects on the serum cholesterol levels following the short-term feeding of tocopherol, pyridoxine, vitamins B_{12}, A, D, and ascorbic acid. In the presence of manifest scurvy, however, the feeding of egg yolk evinces no response until vitamin C has been given. Of the vitamin B group only nicotinic acid appears to produce changes, and in our hands it does not potentiate the fall in levels when fed together with sunflower seed oil.

The changes in serum levels following the feeding of oils and fats are not influenced by whether the basic diet has a very high or very low protein content. Calorie excess in the form of carbohydrate produced marked weight gains with insignificant serum cholesterol level changes (34). These results appertain to serum cholesterol changes. It has been convincingly shown, however, that alteration of the proportion of calories from protein, fats and carbohydrates in the form of a low fat, high carbohydrate diet may lead to a rise in triglyceride

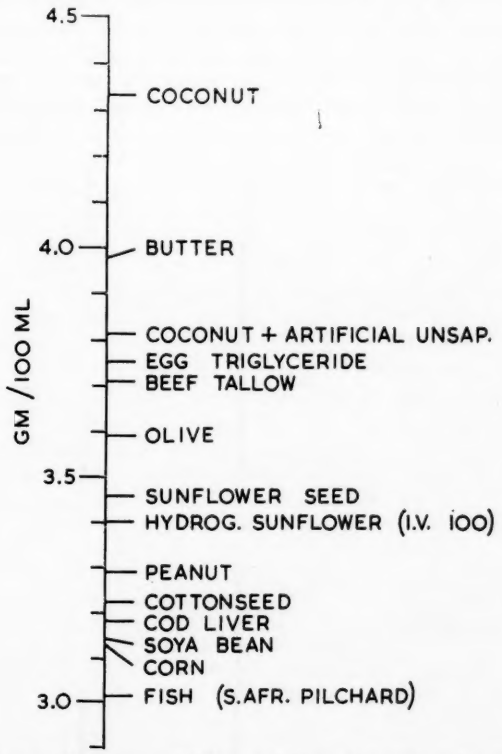

FIG. 2. Percentage solubility of cholesterol in various fats and oils at 37 °C.

levels even though the serum cholesterol falls (1, 6, 41). This rise appears to be transitory. Otherwise the serum cholesterol changes are paralleled by like changes in phospholipid and triglyceride in the unfractionated serum following fat feeding.

Despite individual variations in degree of response, the changes following the feeding of dietary fats and oils have been remarkably consistent in the various centers of the world and on the wide variety of subjects tested. The changes occur promptly, and long-term studies show that their effects are maintained as long as they are fed. Epidemiological studies confirm this. In populations habitually consuming a low fat diet, low serum levels are seen and the converse holds true. While it is true that in the former the prevalence of atherosclerosis and ischemic heart disease is low, it is another matter to assume that the diet is responsible.

DIETARY FATS AND DEVELOPMENT OF ISCHEMIC HEART DISEASE

It was perhaps the sharp fall in mortality in Scandinavia during the 1939–45 war years (57, 80), commensurate with the severe rationing of butter, eggs and cheese, that refocussed the attention of many to the role of dietary fats in the development of the disease. The lion's share of the credit for adding many pieces to the jigsaw puzzle belongs to Keys (47), who pioneered the triple relationship between dietary fat, serum cholesterol level and mortality from ischemic heart disease by his interracial studies. Like all jigsaw puzzles, attempts have been made and will continue to be made to fit pieces that do not belong. Criticism began to be levelled at his hypothesis, in that marked differences in mortality between neighbouring European countries existed without significant differences in total fat consumption. If one bears in mind the different metabolic behaviour of the hard or more saturated fats as opposed to the liquid oils, with respect to the serum lipids at the least, one finds a significant correlation between mortality and saturated fatty acid intake (45) or the dietary hard fat liquid oil ratio (16). Danes, with a high fat intake and low mortality, consumed a great deal of fat in the form of liquid oils, whereas the high mortality of Finns was associated with the fact that most of the relatively low total fat intake was composed of hard fats (see fig. 3).

Genetic factors may govern individual susceptibility, but if one compares the same race under differing environmental conditions one notes the same relationship of mortality to prosperity with its increased dietary fat consumption. Such is seen when one compares the occupational mortality statistics of England (78) with the pattern of food consumption in the different socio-economic classes (63). It is seen too on studying Japanese who have come under the influence of the American way of life in California and Hawaii (49), and likewise Yemenite Jews who have migrated to Israel (84).

The increasing mortality over the last few decades is more easily explained on an increasing fat consumption in the U. S. A. (44) than in Great Britain. Many more subtle changes have occurred however. There has been a decreased overall consumption of the polyunsaturated fatty acids at the expense of an increase in saturated fatty acid consumption. This is seen in a decreased demand

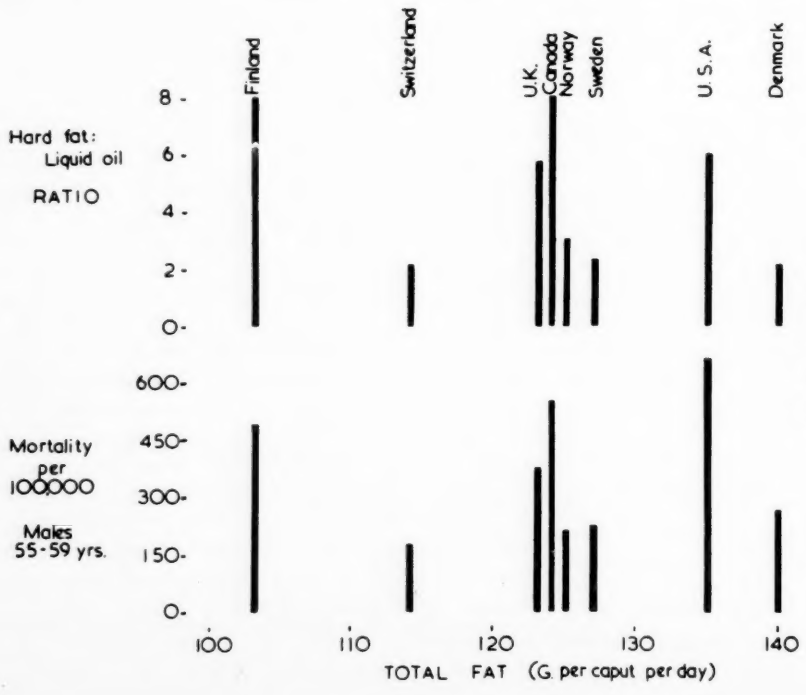

FIG. 3. Qualitative differences in available fat for consumption correlate with different European death-rates from ischemic heart disease better than quantitative differences. Canada and U. S. A. are included on this figure for comparison. (Reproduced from Bronte-Stewart, B. (16).)

for whole-grain cereals and fish and an increasing acceptance of hydrogenated products (63). The composition of animal fat too is dependent upon how the animal is fed (8, 79).

There is finally, amongst the epidemiological features of the disease, the remarkable sex difference in mortality particularly at the younger age groups. When one notes that at given age the American female dies more often than the Japanese male, and that the increasing mortality applies as much to female as male (60), a factor in addition to the sex hormones cannot be denied. This sex difference in mortality is only remarkable in the highly susceptible areas where a great deal of fat is eaten (48, 86), and the possibility could exist that the female has a greater metabolic capacity for handling fat. In the animal this appears to be the case (25). In man, the Framingham study could suggest that males consume considerably more fat than females (59). In addition Bersohn (11) has now shown in long term prisoner studies that the male urinary oestradiol/oestrone ratio remains unchanged on an oil diet but falls to the levels found in patients with ischemic heart disease when butter or hydrogenated fat is the source of fat calories.

It will be seen then that for each of the epidemiological features of the disease an explanation could be found on a dietary fat basis. Unfortunately in most instances other environmental factors could explain these trends. We have yet to discover the ideal, to seek some naturally occurring groups where Nature has performed a good experiment; an experiment in which one variable alone has been altered, the others remaining constant.

In the experimental animal this can be done, and it is pertinent to emphasize that without some form of dietary manipulation involving fats atherosclerosis has not been produced. For various reasons extrapolation of this to man is not wholly acceptable. In man the very nature of the disease precludes such a study of its development. The onset is often insidious and man's life cycle is such that direct observation of the clinical and morbid anatomical course is not usually possible. There is no precise measure whereby one can assess prevalence in the living and in the dead, one is confronted with all the errors inherent in death certification, fashions in diagnosis and terminology.

The approach in ischemic heart disease could be through the study of those diseases, diabetes, myxoedema etc., in which susceptibility is known to be increased. With respect to serum lipid changes at the least it may be more pertinent in some viz. essential xanthomatosis, to study why certain afflicted persons survive. An approach could be made through the controlled clinical trial, although this would be an appraisal of recovery or prevention of recurrence. Two long-term dietary trials (56, 69) report a markedly increased number surviving in the dietary fat restricted group, but neither could claim to fall within the category of the controlled clinical trial. Possibly, the reason why more reports are not available is due to the difficulty whereby only one variable is altered in a dietary trial. Prospective studies are imperative (46) but are likewise difficult as the age at which the stage is set is unknown and factors other than diet undoubtedly enter into the causation of the fully evolved picture. By the age of 20, the serum lipids are different, dependent on race and economic background (17) and a severe degree of coronary atherosclerosis was already present in the young U.S soldiers autopsied in Korea (30).

CONCLUSIONS

Lipid is one of the major constituents of the atheromatous plaque and there is evidence to suggest that it is deposited there. If one accepts that ischemic heart disease results from atherosclerosis, and this has yet to be proven, there is evidence from epidemiological studies to suggest that a disordered lipid metabolism is at the background, and that this disorder can be accounted for by a dietary fatty acid imbalance. Studies on more homogeneous communities, however, are less convincing and, although alterations in the serum lipids can be induced by changes in the dietary fat intake, it has yet to be shown whether or not such alterations have any bearing on the disease.

The implications of the dietary fat hypothesis are wide, affecting developments in animal husbandry, food processing and the health education of our youth. For these reasons it is perhaps reasonable to demand a great deal more factual evidence before widespread application is practised. Nevertheless the dietary fat hypothesis remains a good working hypothesis because, of all the environmental variables that constitute our present day mode of life, no other at present known can be so closely related to pathologic, metabolic and epidemiologic features of the disease.

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Pathology of lipid disorders: liver and cardiovascular system[1]

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THE ASSIGNED SUBJECT—Pathology of Lipid Disorders—is too large to be encompassed within the available space and time. With our Chairman's permission, I have restricted this communication to only some aspects concerning liver, heart and vessels. We shall make no pretense of attempting to review the presently available knowledge of the effects of abnormalities of fat transport and metabolism on these organs, but confine this report to recent observations made in our laboratory by our colleagues, Robert M. O'Neal, W. A. Thomas, Eduardo A. Porta, Joe W. Grisham, John S. Meyer, H. Imai, M. Suzuki, and the writer. The justification for taking liver and cardiovascular system together rests not only on our interests in these twin areas, but also on the phenomenon, which we hope to demonstrate, that in at least some experimental dietary situations both are dramatically altered and often simultaneously.

Morphologic manifestations of lipid disorders of a wide variety of etiologic and pathogenic types are probably observed more frequently in the liver than in any other organ. Excesses or deficiencies of caloric intakes as well as a spectrum of imbalances and deficiencies of essential food factors, including several amino acids and vitamins, result in an abnormal accumulation of fat within the liver. In several well documented instances the excess of fat is associated with eventual damage to the parenchyma producing frank cirrhosis and even primary cancer. Dietary choline deficiency has been studied more intensively in animals than have other known lipogenic factors. Yet, though well over a quarter of a century has elapsed since the discovery of the lipotropic action of dietary choline by Charles H. Best and his group in Toronto (2), its mechanism of action still awaits elucidation. We have not answered this problem, but have attempted to obtain leads in this direction by studying the earliest stages in the appearance of hepatic fat in choline-deficient animals.

Dr. Porta while in our laboratories, using mensurational microscopy, discovered it was possible to observe and measure (20) the appearance of histochemically demonstrable fat in liver cells of normal animals within 1 hour after corn oil-gavage. In suitable controls the amount of fat that appeared in centrolobular areas after this procedure was significantly decreased by administration of choline chloride with the oil (fig. 1). We concluded that a relative deficiency of choline had been produced by feeding fat alone. This swiftly developing deficiency had been manifested by the deposition within 1 hour of measurable amounts of stainable lipid in centrolobular cells. Not all the administered fat was absorbed within the 1-hour period and after 4 hours, as more reached the liver, it again appeared unless a second dose of choline was administered 3 hours after the initial gavage (fig. 1). These findings indicate that to prevent the abnormal appearance of fat in these livers exogenously administered choline must reach the parenchymal cells at the same time as does the absorbed fat. These experiments are being extended, substituting alcohol and sucrose for the fat. Apparently the abundant stores of choline, either free or bound, in the animal's tissues are ineffectual in preventing ingested fat from accumulating in abnormal form in liver cells. In this regard, the lipotropic agents appear to occupy a unique position in experimental nutrition.

These studies and others (1) directed our attention to the possibility that this prompt manifestation of choline deficiency might well be concerned with oxidative enzymes of the mitochondria. We have therefore studied mitochondria with the electron microscope in various stages of choline deficiency. Again using, as a model, livers of rats fed a moderately choline-deficient diet for only a short period (4 days), Porta (21) while in St. Louis applied mensurational techniques to outlines of mitochondria of centrolobular hepatic cells of these animals and suitable controls (those fed the basal diet supplemented with choline and those fed a stock laboratory diet (Purina chow)). Most hepatic mitochondria have an oblong or oval profile in electronmicrographs but their shapes in centrolobular liver cells of animals fed the choline-deficient diet for 4 days became rounded in outline (figs. 2, 3). Statistical evaluation of the ratios of oblong to round mitochondrial profiles in the 2 groups

[1] Original experiments described in this paper were supported in part by grants-in-aid of research by Public Health Service Grants H-1968, C-2548 and CRT-5017; the Nutrition Foundation, Grant 269 and the Lipotropic Research Foundation.

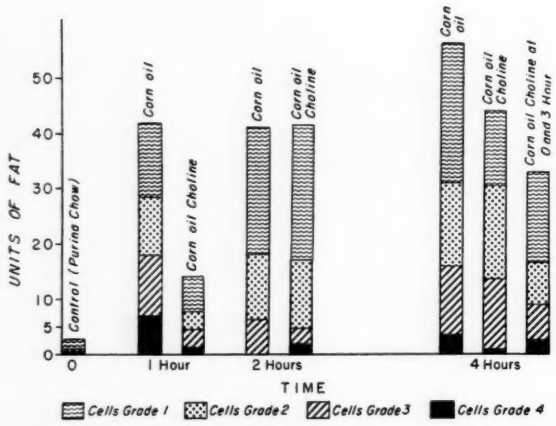


FIG. 1. Effect of feeding corn oil with and without a choline supplement on centrolobular stainable fat in livers of rats over a 4-hour period. Units of fat represent a histochemical method of estimating the amount present (20). At the end of 1 hour after gavage the choline supplement greatly decreased the amount of fat found in the liver. But after 2 hours and 4 hours, choline had lost this effect on fat that continued to be absorbed from the gastrointestinal tract and brought to the liver. The choline effect could be again demonstrated by giving another dose 3 hours after gavage and killing the rats 1 hour later (last column on right).

confirmed these observations (table 1). Choline supplementation of the basal diet fed the controls was effective in preventing this alteration to a significant degree but not as completely as did the stock diet of laboratory chow fed a second control group of rats.

In later stages of choline deficiency at the time when the fatty livers are becoming fibrotic and cirrhotic, this alteration in shape of mitochondria of lipid-laden parenchymal cells is complete because all of them have become round in profile (fig. 4). Electronmicrographs taken by my colleague, J. W. Grisham, clearly demonstrate not only this point, but also even more dramatically the striking increase in size of these mitochondria (fig. 5). It appears then that the alteration in mitochondrial form observed and measured by Porta in the early stages of choline deficiency are continued throughout later stages and progress to gross enlargement. We regard the change from oblong to spherical shape as the initial step in mitochondrial enlargement induced by choline deficiency. Comparably early or consistent alterations in choline-deficient livers cannot be demonstrated to affect ergastoplasm, Golgi networks or other cellular organelles of either cytoplasm or nucleus. But the mitochondrial alterations are a cardinal patho-electronmicroscopic change of choline deficiency and may indicate that the site of the lipotropic action of dietary choline may involve either the entire mitochondrion or its surface. Certainly, choline is necessary for the maintenance of the normal structure and function of hepatic mitochondria. This hypothesis does not, of course, exclude the importance of other dietary factors for normal mitochondrial function.

Part of the evidence linking choline deficiency so selectively with enlargement and rounding of the mitochondria rests on the fact that in the same electronmicroscopic section in which parenchymal mitochondria attain such bizarre proportions, mitochondria of adjacent reticulo-endothelial and ductular cells are not similarly affected (fig. 4). This fact would appear to exclude the possibility that fixation artefacts are responsible in any

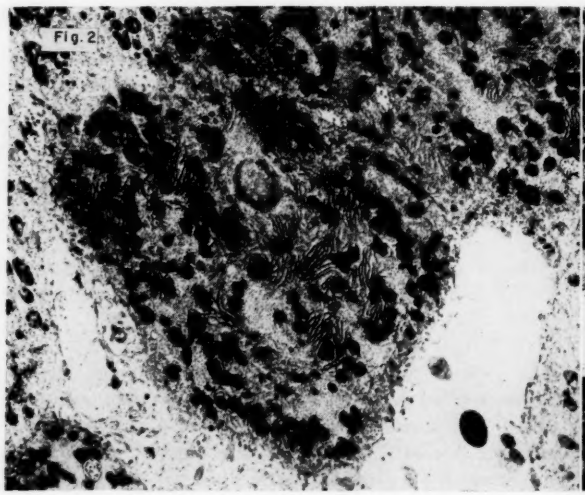


FIG. 2. Liver cell under electron microscope illustrating that most mitochondria possess small oval shapes in the normal control animals. (Magnification approximately × 2600.)

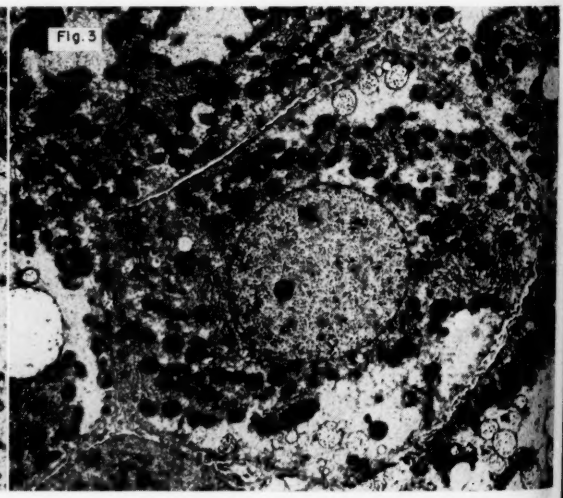


FIG. 3. Liver cell under the electron microscope from rat fed a moderately choline-deficient diet for 4 days which illustrates that an increase in mitochondria with round outlines has occurred when compared with the normal (fig. 2). See table 1 for statistical evaluation of ratios of oval to round mitochondria in these animals. (Magnification approximately × 2600.)

way for the unusual appearance of parenchymal mitochondria in these choline-deficient animals' livers. Mitochondria in livers of controls pair-fed the basal diet supplemented with choline remained entirely normal (small and oblong in outline). Furthermore as will be shown below, mitochondria in fatty livers induced by means other than choline deficiency do not become similarly swollen and round. Large spherical mitochondria in the cells of choline-deficient rat livers cannot be explained as a secondary effect of either fixation or abnormal fat, but appear to be the direct effect of lack of choline. Furthermore, restoration of choline to the animals' diet promptly restores the normal small, oblong outlines to the mitochondria despite the fact that the abnormal parenchymal fat may persist for some further time before its mobilization.

The pathogenesis of cirrhosis in choline-deficient rat livers has been studied in our laboratories and the results reported elsewhere (7, 12). Current studies with the electronmicroscope have resolved the controversy concerning the nature of so-called 'oval cells' which infiltrate between the fatty cysts. Under the electron microscope these cells are clearly ductular epithelium possessing microvilli and all other characteristics of the cells in walls of normal hepatic cholangioles (fig. 4) (19). Other studies by my colleagues Grisham and Porta which cannot be detailed here indicate that these cells are formed from normal ductular cells and not from altered or abnormal parenchyma.

Until recently it was the consensus of experimental pathologists who had studied dietary cirrhosis that the type corresponded to nutritional or monolobular Laennec cirrhosis seen in man and described in detail by many morbid anatomists but most recently by Gall (4). But we have observed frequently that in the late stages of hepatic injury in rats fed choline-deficient diets for

TABLE 1. *Ratios of Oblong to Round Profiles of Mitochondria in Electronmicrographs of Choline-Deficient Rats and 2 Groups of Control Animals*

Group	Total Area Mitoch.	Oblong Mitoch.	Round Mitoch.	Ratio Obl./Round	
I	2.690±0.283*	2.110±0.315*	0.575±0.063*	2.257±0.527*	P<.05
II	3.062±0.583*	1.593±0.182*	1.472±0.843*	0.790±0.243*	NOT SIG. P<.01
III	2.388±0.193*	0.999±0.098*	1.387±0.125*	0.413±0.003*	

Values for group I are from animls fed a normal stock commercial laboratory diet (Purina chow); group II from animals fed a semi-synthetic diet deficient in choline; and group III from animals fed the same choline-deficient diet supplemented with choline. All differences between the 3 groups in regard to ratios of oblong to round mitochondrial profiles in electronmicrographs are statistically significant.
*Standard error of the mean.

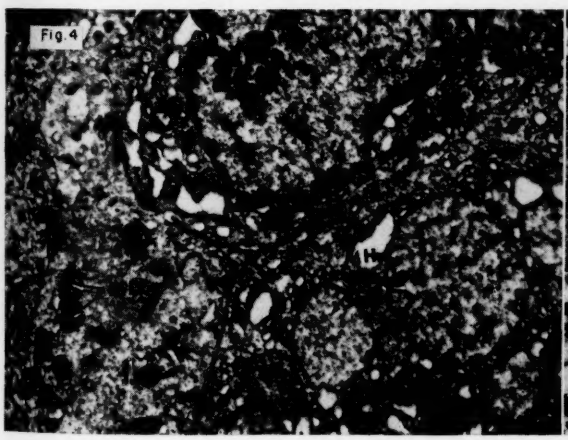


FIG. 4. Electron micrograph of several cells in the liver of rat fed a choline-deficient diet for several weeks. Mitochondria of parenchymal cells (MH) are greatly swollen, round and crowded together while those in the adjacent ductular cells (MD) are unaltered thereby indicating the adequacy of fixation. (Magnification approximately × 7,600.)

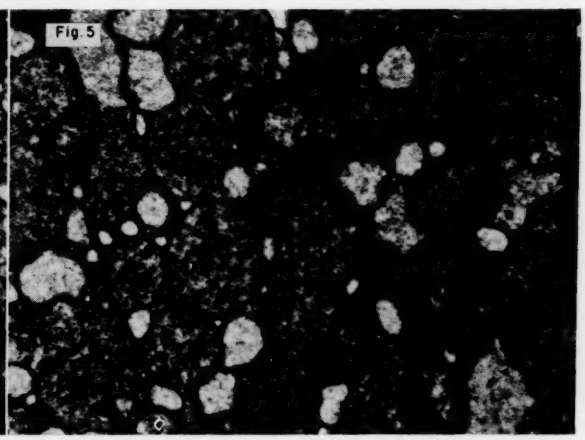


FIG. 5. Detail of parenchymal cell of type shown in preceding figure. Crowding, swelling and rounding of the macromitochondria are well shown. (Magnification approximately × 8,275.)

periods of a year or more, and within even shorter periods if the animals are treated with choline for a few weeks before autopsy, a metamorphosis of the type of cirrhosis transpires (10). In these cases the monolobular nutritional type of cirrhosis changes its form so that it more closely resembles postnecrotic cirrhosis in man (figs. 6, 7). The alteration results from uneven growth of regeneration nodules formed during the initial period of the experiment. Nodules which contain the least fat or that lose their abnormal lipid most rapidly grow faster than internodular parenchyma and smaller adjacent fatty nodules. The latter are compressed to atrophic remnants leaving broad scars between the greatly enlarged nodules. These macronodules develop multilobular structures indicated by the numerous central and portal areas that appear within them. The 2-celled liver plates formed during active regeneration eventually achieve an adult unicellular form. The resulting picture is therefore one resembling the conventional postnecrotic cirrhosis in that broad bands of stroma course irregularly between large units of normal appearing liver tissue (now devoid of stainable fat and hyaline) comprised of many subsidiary lobules. Pathogenic studies at intervals during the experimental period (5 months of choline deficiency followed by 3 months of complete diet) established however that frank necrosis did not develop at any stage. Hepatic destruction proceeded always by the process of atrophic compression and withering of parenchyma overburdened with abnormal amounts of fat (12). The term 'postnecrotic' for the eventual lesion attained, although descriptively apt is unsuitable on a pathogenic basis. We have therefore introduced the term 'metanutritional cirrhosis' to describe this late stage and indicate the alteration in form, 'meta-', and at the same time the etiologic mechanism. Evidence has been reported (22) that a similar sequence of events (nutritional into metanutritional) may occur in livers of alcoholic man, particularly in individuals subjected to contemporary therapeutic procedures for this condition. We have seen serial biopsies from alcoholic patients demonstrating this same transition from nutritional to metanutritional cirrhosis in excellently documented material obtained by G. Klatskin of Yale Medical School (personal communication). This concept may help explain otherwise paradoxical cases of 'postnecrotic' cirrhosis found at autopsy in alcoholic individuals in which evidence for acute or subacute hepatitis of either viral or toxic origin is completely lacking.

Fatty livers are frequently encountered both in biopsies and at autopsy of obese individuals (23). We do not know why fat people have fatty livers and although this question has stimulated some of our experimental studies in animals, the latter have not elucidated the mechanism. Several interesting observations from these studies in rats and mice performed by my colleagues, John S. Meyer and H. Imai have emerged, however. Abnormal fat deposited in livers of rats has been consistently observed when their caloric intakes have been elevated either by repeated administration of small amounts of insulin twice daily (15) over a period of several weeks, or in rats in which polyphagia was induced by damage to the hypothalmus following stereotaxic acupuncture (14). Stylostixis resulted in a more extreme form of obesity and fatty liver than did insulin administration, but results were qualitatively similar. Abnormal accumulation of fat produced by hyperalimentation differed from that associated with dietary choline deficiency in several significant ways. Neither additional dietary choline nor supplementation by more protein (casein) prevented the accumulation of hepatic lipid in the obese animals. The fat was strikingly periportal in its lobular position (figs. 8, 9), whereas in choline deficiency it was

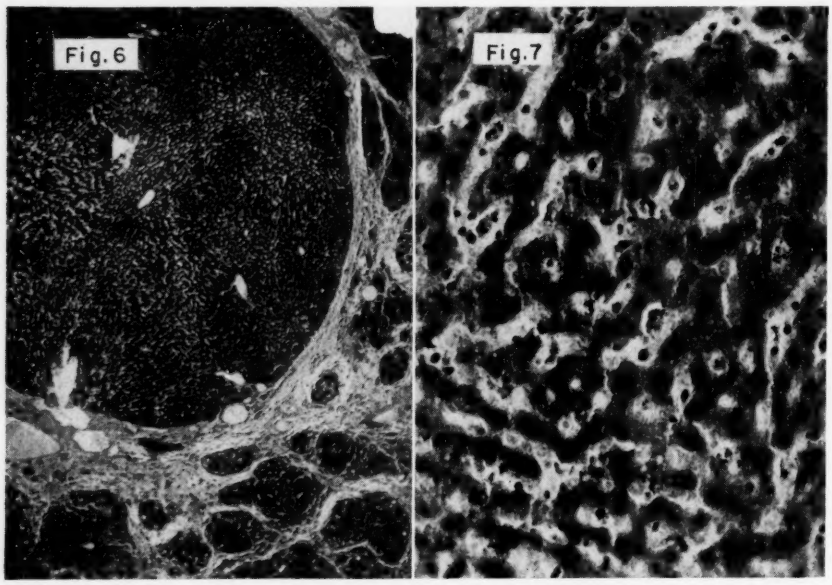


FIG. 6. Low power photomicrograph (× 30) of metanutritional cirrhosis produced in a rat as described in text. A large macronodule contains multiple lobular units as indicated by the numerous central and portal spaces within it. Surrounding internodular tissue and small nodules are compressed and undergoing atrophy leaving wide trabeculae.

FIG 7. High power (× 300) of the large nodule shown in preceding figure to illustrate conversion of 2-cell thick plates (upper right) to mature 1-cell thick plates (lower left). Eventually all 2-cell thick plates mature to adult form.

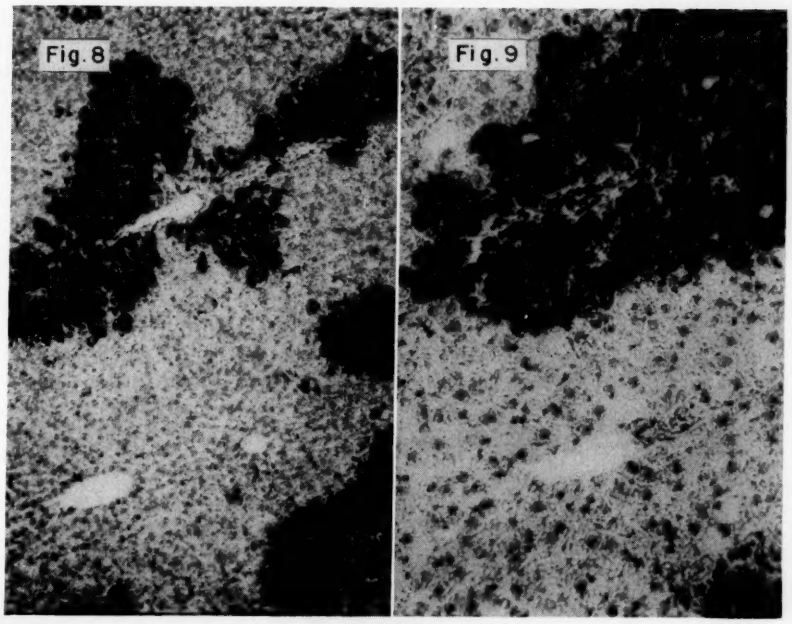


FIG. 8. Strikingly concentrated periportal massing of intracellular fat (black) is illustrated in this section of a fatty liver induced by polyphagia following hypothalmic injury to a rat. A centrolobular area (completely devoid of surrounding fat) is shown in lower left. × 30.

FIG. 9. Higher magnification of an area from the section illustrated in preceding figure. Portal triad within the fatty periportal cells can be clearly seen. × 300.

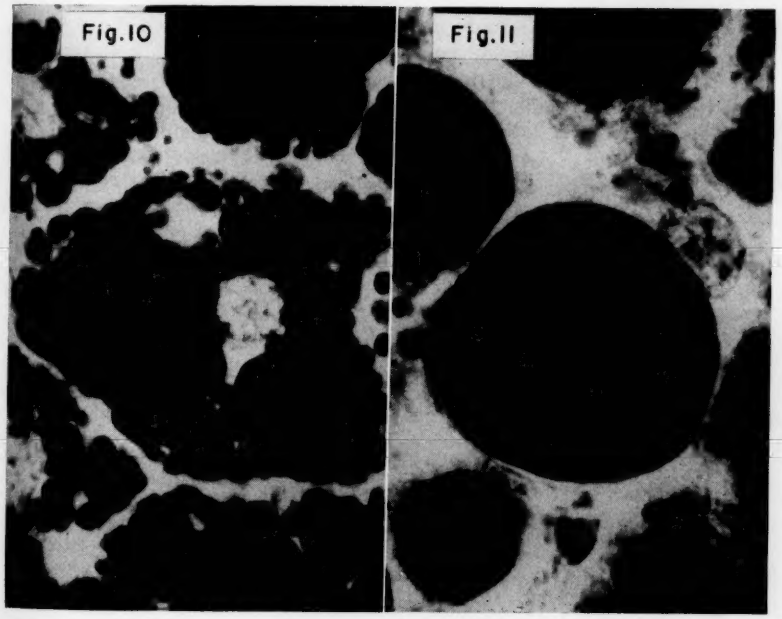


FIG. 10. Oil-immersion photomicrograph of a periportal fat-laden cell from a polyphagic rat. Fat is held within small intracytoplasmic compartments and does not fuse into large globules as in choline deficiency (see fig. 11 below). Nuclei (pale areas) are not displaced. × 1,000.

FIG. 11. A single cell from a choline-deficient animal illustrates the manner in which in this condition the abnormal fat forms a large, single intracytoplasmic spherule which displaces the nucleus to one side (upper right of cell). × 1,000.

initially centrolobular. Under higher magnification (figs. 10, 11) fatty liver cells of the obese animals contained myraids of small fat droplets which neither displaced their nuclei nor deformed their outlines from classic hexagons, although the cells were greatly enlarged. In choline deficiency, on the other hand, large globules of fat—usually only 1 or 2 per cell—displace the nucleus to one side and by distension deform the cell to a sphere with consequent loss of normal hexagonal form (fig. 11). By electron microscopy (fig. 12) it was further discovered that the shape and size of the mitochondria in the fatty liver cells of the obese rats were not altered; an observation in striking contrast to that of the large spherical forms assumed by the mitochondria in choline deficiency (fig. 5).

An appreciable percentage of mice given gold thioglucose become obese, and Meyer, Imai and Scott (16) in our laboratories have studied these livers which also

become fatty. The cytologic features of this fatty liver by both light and electron microscopy (figs. 13, 14) resembled those of the obese rats described above, and lacked the large intracellular lipid globules and round macromitochondria of choline deficiency. But the distribution of the fat was centrolobular (fig. 13). Our data do not indicate whether this latter difference is the result of the method of inducing the obesity or to the difference in species employed. Significant accumulation of centrolobular fat in the mice was associated with obesity rather than the administration of the drug because abnormal lipid was found only in livers of the animals that became obese (body weights of 36 gm or more). Fatty livers did not develop in those resistant animals which despite single or repeated doses of gold thioglucose failed to become obese. In all these investigations involving both rats and mice generous supplements of dietary choline (1.0%) did not prevent the livers becoming fatty.

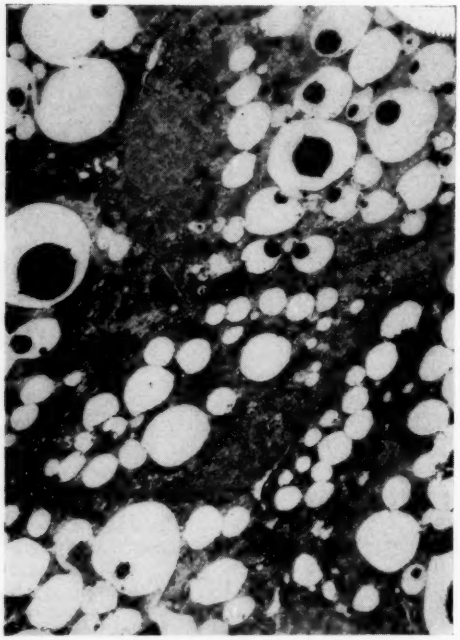


FIG. 12. Electron micrograph of periportal lipid-laden cells from a polyphagic rat to illustrate the small sacs in which lipid droplets are individually held and the small, oval mitochondria which are in striking contrast to the large, prominent round mitochondria of choline deficiency. × 2,300 (approx.).

Recently, Wilgram and Ingle (28) have reported the development of fatty livers in rats in which obesity was induced by forced feeding by tube. These investigators considered their diets normal (although they contained a high percentage of fat) and concluded that their data supported the concept that excessive caloric intake results in fatty livers. Unfortunately, their studies did not indicate which lobular zone of their animals' livers was initially affected (central or portal) and they did not include a force-fed control group in which additional supplements of choline were incorporated in the diet.

From these studies of hyperalimentation we are unable to explain the mechanisms involved in the production of the consistently associated abnormal accumulation of hepatic lipid. Neither lipotropic nor protein supplements prevented the development of fatty livers in our animals as long as the caloric intake was in excess of that of normal controls fed the same food mixtures ad libitum. Fat animals as well as fat people do develop fatty livers, and reduction of caloric intakes is the only prophylactic or therapeutic procedure thus far demonstrated that will prevent or remove this type of excessive hepatic lipid.

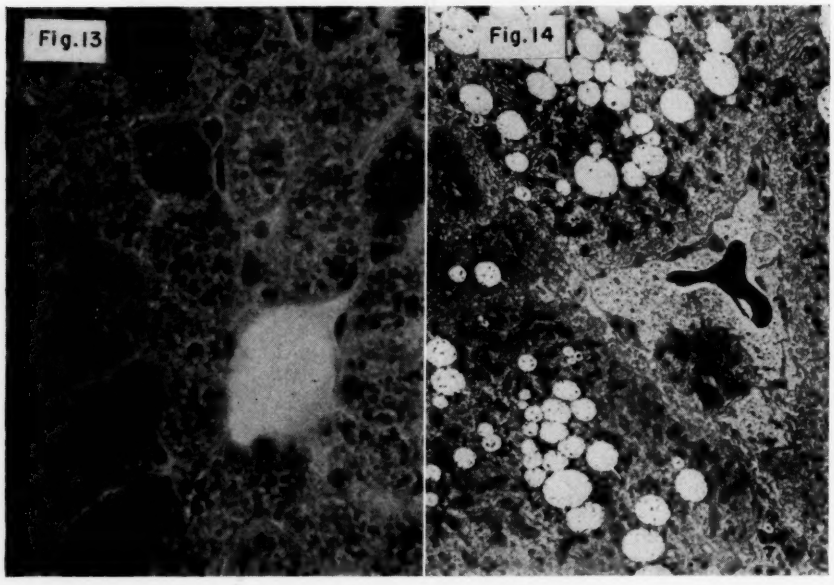


FIG. 13. Lipid-laden pericentral cells from the liver of a mouse in which obesity had followed the administration of gold thioglucose. The fat is held within small droplets and neither fuses together into large spherules nor displaces the nuclei as in choline deficiency. A central vein occupies the lower right. × 710 (approx.)

FIG. 14. Electron micrograph of a cell similar to that shown by light microscopy in the preceding figure. Note resemblance here to the electronmicrographic appearance of fatty liver cells in the polyphagic rat shown in fig. 9. Mitochondria are small and oval; the fat (much has dropped out) is held in small individual sacs. × 1800 (approx.)

TABLE 2. *Composition of Diet Referred to as Basal Myocardial Infarction-Producing Diet*

	*
Cholesterol	5%
Propylthiouracil	0.3%
Sodium Cholate	2%
Choline Chloride	0.2%
Salt Mix	4%
Vitamin Mix	2%
Alphacel	6%
Casein	20%
Sucrose	20.5%
plus Butter	40.0%
	100.0%

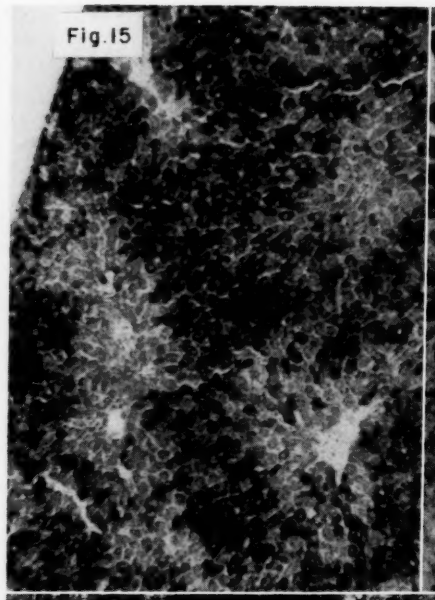
* Percentages represent the percentage-weights of the various ingredients of the total diet. Male Wistar albino rats (starting weight about 100 gm) fed this diet for 5 months develop extreme hypercholesterolemia and arterial thromboses with myocardial and/or renal infarcts. Infarcts occur in one third of the animals, but do not appear until the third month on the diet.

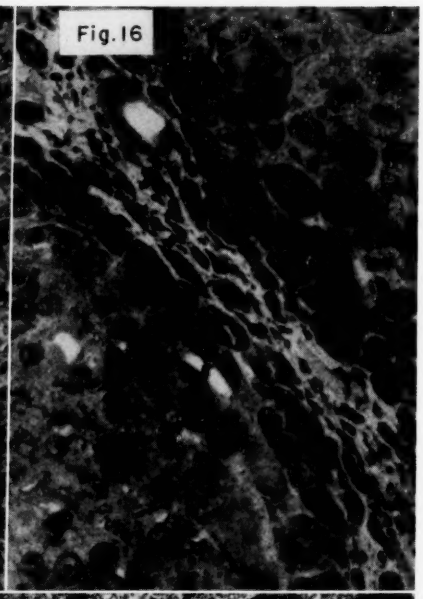
Probably the most extreme form of fatty livers that we have encountered has been in rats fed a diet (table 2) (8) designed to produce coronary arterial thromboses and myocardial infarcts. This food mixture contained 40% saturated fat (butter), 5% cholesterol, 2% bile salt (sodium cholate) and 0.3% thiouracil. When it was fed to male Wistar rats (initial weights 100–110 gm) for periods of 2 to 4 months, their livers became so fatty they were grossly soft, putty-like and almost white. The fat is initially periportal (fig. 15) but soon distends almost every cell. Those at the periphery of the swollen lobules become compressed, atrophic and eventually disappear leaving cellular remnants, extracellular fat and condensed reticulin which forms annular periportal trabeculae. Proliferation of cholangioles in these regions add to an appearance reminiscent of a primary type of biliary cirrhosis (fig. 16). Here again, electron microscopy (fig. 17) revealed that the mitochondria were not swollen and rounded to the extent seen in choline deficiency. As might be expected from all these morphologic differences between the cholesterol-induced fatty livers and the hypolipotropic ones, high dietary supplements of choline chloride (1.0%) failed to prevent the abnormal deposition of lipid, including cholesterol, in the livers of animals fed the 'myocardial infarction-diet'.

Development of cirrhosis in these animals as well as in the choline-deficient rats supports the concept to which we have adhered for some years,—that excessive loading of liver cells by abnormal lipid leads to their distension, cellular disruption, fatty cyst formation, degeneration and death. Cell-loss occurs in this sequence not by frank necrosis as in viral or toxic conditions, but by a form of compression atrophy and 'withering away' associated with overloading by fat. Others have attempted to implicate alternate factors such as protein imbalances, directly harmful effects of choline deficiency on the cell or ischemia induced by sinusoidal compression. We believe the data obtained from our laboratories increasingly support the 'lipid loading concept' of cirrhogenesis, and the recently reported experiments of Wilgram and Ingle (28) offer strong confirmation. In their studies a normal diet was force fed so that amino acid imbalances or food factor deficiencies are unlikely mechanisms. In the resulting fatty livers they obtained cirrhosis of an advanced nature despite the apparently normal quality of the diet employed. Cirrhosis in their rats was most likely the result of extreme lipid loading of liver cells induced by the excessive caloric intakes imposed on the force fed rats. We suggest that in both animals and man the production of exceedingly fatty livers by a variety of mechanisms results in lipid loading that at least sets the stage for the onset of architectural destruction of relatively irreversible nature culminating in cirrhosis of various types. The end result, in choline deficiency at least, may be so grossly altered in form that it eventually resembles postnecrotic cirrhosis—the type for which we have suggested the term 'metanutritional cirrhosis.'

The cardiovascular system is no less important than the liver in a consideration of the subject of pathology of lipid disorders. In the paper preceding this one, Bronte-Stewart considered the vessel wall (atherosclerosis) in relation to lipids, and I do not wish to infringe on his subject. But in our laboratories R. M. O'Neal, W. A. Thomas, M. Suzuki and I have been concerned for the past few years with another aspect of the cardiovascular system in relation to lipid disorders: namely the production of occlusive arterial thromboses in hearts and kidneys of experimental animals by dietary means alone (table 2) (11, 13). The thrombi which have been produced are found predominately in coronary and renal arteries. They resemble those encountered in man, and their appearances similarly depend upon the length of time elapsing between their formation and death of the animal (figs. 18, 19, 20). In recently formed thrombi, lines of Zahn are prominent, but within a short time invasion by fibroblasts and macrophages produces a picture of organization. Although our evidence is incomplete, we believe these invading cells may derive from vascular endothelium. The presence of large mural thrombi (fig. 21) within the left ventricular chamber is a frequent occurrence in the hearts in which infarcts are found. Intraventricular mural thromboses of this type have not been found associated with infarcts produced in this and other laboratories by simple surgical ligation of rats' coronary arteries. These findings provide further support for our concept that the dietary regimen profoundly alters thrombotic and thrombolytic mechanisms in these rats.

Further examples involving the vessels of the thyroid and other organs have been found in rats fed this basal 'myocardial infarction producing' diet (18). But the most important effect of the form of lipid disorder produced by this diet is on the thrombotic and thrombolytic mecha-





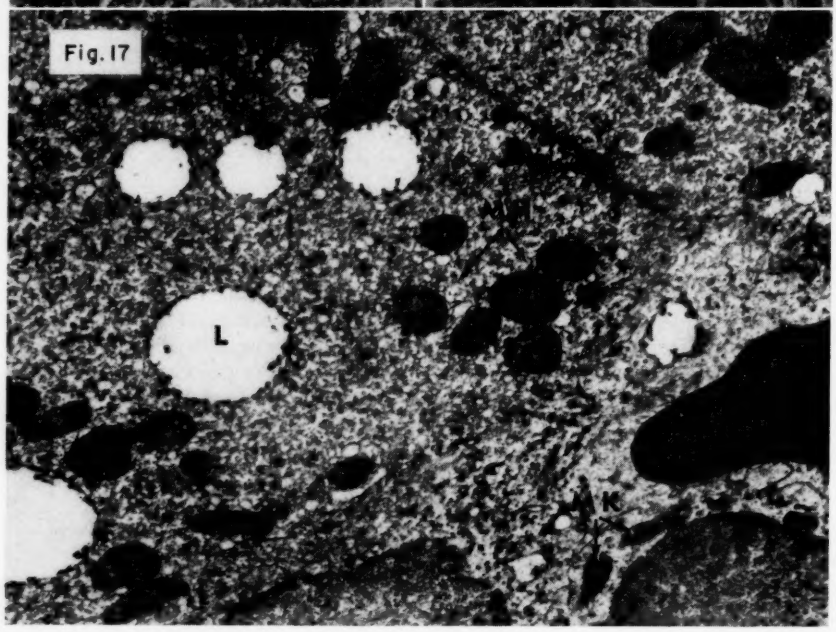


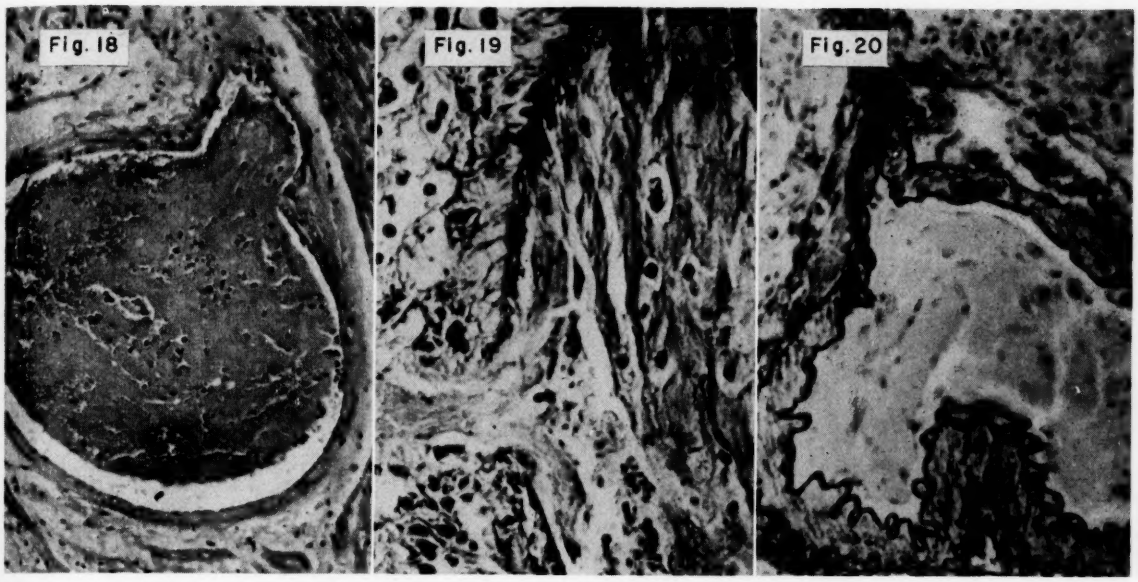
FIG. 15. Liver of rat fed the diet described in table 2 for a period of 2 weeks. In the frozen section stained for fat (Oil red O) (fat appears black) the lipid-laden cells are periportal. Several central veins stand out because haloes of fat-free parenchyma surround them. × 30 (approx.)

FIG. 16. As cirrhosis develops in these livers of cholesterol-fed (table 2) rats, lipid-laden cells in periportal positions become compressed and atrophic as nodules of regenerating liver enlarge. Stages of compression and atrophy are shown in this trabecula running from upper left to lower right and small bile duct proliferation is present. × 200 (approx.)

FIG. 17. Electron micrograph of lipid-laden cells in rats fed the cholesterol-containing diet of table 2 (L—lipid). Mitochondria of hepatic cells (MH) have not assumed the large, swollen round appearance typical of those in the choline-deficient parenchymal cell. Mitochondria in Küpffer cells (MH) are normally smaller than those in parenchymal cells. × 10,000 (approx.)

nisms of the blood. Additional evidence to support this view has been provided in this laboratory because we have demonstrated with the thromboelastograph (27) extreme prolongation in the time required for fibrinolysis of clotted blood obtained from rats fed the myocardial infarction-diet. With blood obtained from rats fed a modification of this diet in which corn oil had been substituted at the 40% level for the butter, fibrinolytic times were within the same range obtained with blood from rats fed a complete semi-synthetic food mixture. Thromboses and infarcts did not develop *in vivo* in any of the rats fed the corn oil-modification of the basal diet or in any of those given the normal semisynthetic food mixture (11).

The myocardial and renal infarcts (figs. 22, 23, 24) resulting from the ischemia produced by occlusive thromboses in coronary and renal arteries are associated in these experiments with only simple lipoidosis of the walls of the vessels rather than true intimal atheroma of the type Bronte-Stewart discussed. Indeed, when the basic dietary regimen (table 2) is manipulated by removal of the thiouracil so that the animals survive for

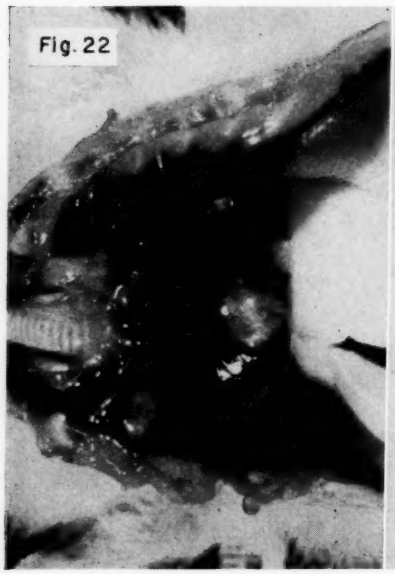


FIGS. 18, 19, 20. These figures all illustrate types of arterial thrombi encountered in vessels of heart and kidney of rats fed the basal 'myocardial infarction producing' diet of table 2. Thrombi in figs. 18 and 19 are within coronary arteries and show different degrees of organization; that in fig. 20 is in a renal artery. Regions of heart and kidney supplied by these vessels in each case had undergone infarction of type shown in figs. 22, 23 and 24. × 95, × 425, and × 225, respectively.

FIG. 21. Gross appearance of a large mural thrombus within the cavity of the left ventricle of a rat fed the basal myocardial infarct-diet. Frequency and size of such thrombi in these rats emphasize the ease with which thrombi form and persist in these animals.

periods longer (up to 1 year) than that of only the 2 to 4 months when fed the basal diet, infarcts are few but mural arterial lesions are numerous and advanced even to the stage of true plaque formation (17). This relative, albeit not complete, dissociation of atheroma from occlusive thromboses with infarction in experimental animals may have its counterpart in man if current epidemiologic studies continue in their present trend of results. Atheroma may be a pathogenic factor of some importance in arterial thromboses and infarction, but the occurrences of the 2 conditions may not be related at all or at best only by chance. Until evidence to the contrary is at hand, we believe progress in understanding heart disease may be more rapid if this hypothesis is borne in mind.

Partly because of the intensive clinical preoccupation with levels of serum lipids, particularly of cholesterol, in patients with myocardial infarction, we have obtained similar data from our animal experiments (24, 25). In many respects the results are quite analogous to those for man. In repeated experiments (26) one-third of the rats surviving this basic dietary regimen for 6–8 weeks developed thromboses and infarcts during the third and fourth months of the experiment. But two-thirds fed exactly the same food-mixture failed to manifest any evidences of arterial thromboses even on gross and microscopic examination at autopsy. Although the lipid fractions (total lipids, total cholesterol, esterified cholesterol, free cholesterol, phospholipid, triglyceride) measured in



FIGS. 22 and 23. Gross appearances of 2 representative infarcts, 1 of a heart and 1 of a kidney, produced by arterial thromboses (figs. 18, 19) in rats fed the basal myocardial-infarct diet. Whether a myocardial infarct is large or small, it nearly always involves the cardiac apex.

FIG. 24. Low magnification-photomicrograph to illustrate the distribution of a typical myocardial infarct produced by coronary arterial thromboses induced by feeding the rat the diet described in table 2. The infarcted muscle (dark in photograph) involves the anterior wall of the left ventricle continuing down and around the apex and up into the intraventricular septum for about one-third of its length. × 6 (approx.)

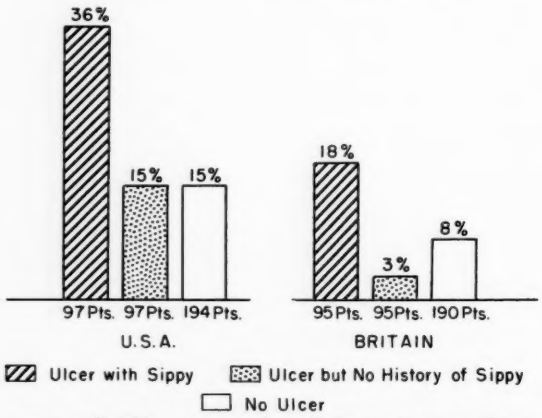


FIG. 25. Graphic representation of the percentages of myocardial infarcts found at autopsy in patients with peptic ulcers treated with the Sippy-type dietary regimen; in those with ulcers only (without dietary treatment), and in controls selected to be comparable in age, weight, sex, place and time of autopsy. Data are presented separately for the 2 countries, America and England. Note that over twice as many infarcts were present in both countries in those patients with ulcers treated by Sippy diets than in either of the control groups. Difference in the incidences of infarcts in the English group of ulcer patients without dietary therapy is not statistically significant from that for the controls in England (neither ulcer nor therapy). Note the over-all lower incidence of infarction in England compared with United States.

the sera of rats fed the basal diet and that developed thromboses and infarcts were greatly elevated, none rose higher or more quickly in these animals than in the sera of the rats fed the same diet but which failed to develop thromboses and infarcts. These results indicate clearly to us that some constituent of the serum or blood other than those above must be found and measured to provide the correlation we seek with the onset and extent of thromboses. We are continuing our search and should we be successful with our animals, it will then be possible to hope the results might be diagnostically applicable to man.

The diet employed in these experiments is admittedly unphysiologic and a far cry from that ever consumed by man. But it produces lesions (thromboses and infarcts of hearts and kidneys) in the animals within a few months that takes years to develop in man. We defend its use on these grounds. But the therapeutic dietary regimen (Sippy) used in some cases for the treatment of peptic ulcers is high in saturated fats and contains appreciable amounts of exogenous cholesterol. Many of these patients remain on this diet for long periods—up to years. It seemed pertinent to examine the incidences, at autopsy, of coronary arterial thromboses with resultant myocardial infarction in patients with peptic ulcers, in this country and in England, treated by these regimens (3). Control patients in whom ulcers were found at autopsy but who had been treated by means other than dietary were similarly studied. Additional control patients of similar ages, weights and sexes were selected from the same autopsy series' for the same time-periods whose histories failed to indicate the presence of peptic ulcers during life, and in whom neither duodenal nor gastric ulceration was encountered at autopsy. The results are presented in graphic form in figure 25. Over twice as many myo-

cardial infarcts were found at autopsy in those patients of both the American and English series' that had received the Sippy-type diets than were found in patients of either the control group with ulcers (but no history of dietary treatment), or in the group without ulcers. The all-over low incidence of infarcts in the English patients confirms other studies indicating that cardiovascular disease in America is higher than in the British Isles. It is significant however that the ratios of the incidence of infarction in the patients treated by diet to that in either type of controls are identical in the 2 countries, being over twice as great in the former.

Many other tissues and organs in addition to livers, hearts and vessels in animals and man are profoundly altered in lipid disorders. No attempt has been made to discuss these pathologic changes here. Probably consideration of adipose tissue itself constitutes the most glaring omission from this paper, but the available knowledge of fundamental pathologic changes in fat depots associated with abnormalities of fat absorption, transport, metabolism and storage is paradoxically scant. The kidney is often involved; for example it may undergo extreme hemorrhage and necrosis in acute stages of choline deficiency (5, 6) which develop within only a few days when young rats are fed a hypolipotropic diet. Survivors of this type of bilateral renal cortical necrosis of proximal convoluted tubules may develop severe degrees of hypertension in later adult life even if maintained on completely normal diets during their entire lives except for this brief period of choline deficiency at weaning or soon after (9). A chronic form of renal damage resembling some forms of glomerulonephritis is produced by dietary choline deficiency if the animals are young adults at the beginning of the experiment (6). Both forms of renal injury (acute in weanlings: chronic in young adults) appear to result from 'lipid loading' of the epithelial cells of the proximal convoluted tubules. Rapid loading in the young leads to necrosis. The slower rate of lipid loading which occurs in older rats is associated with the chronic form of tubular atrophy, interstitial reticulin condensation and glomerular fibrosis. It is our belief that the pathogenesis of both forms of dietary renal injury and hypertension are further examples of the injurious effects of extreme deposition of abnormal parenchymal fat analogous to the fibrosis and cirrhosis following hepatic 'lipid loading.'

It is currently popular to suspect the quantity and kind of dietary fat consumed by man of being the etiologic culprits for the high incidences of a number of important diseases rampant in populations of the western world including heart disease, cirrhosis, diabetes and even cancer. These hypotheses are stimulating to the clinical investigator and basic medical scientist alike, but applications of theories to the extent of drastically altering our dietary habits must await that time when every reasonable doubt of the truth of these concepts has been removed. That day is far off. Meanwhile, established principles of good nutrition should prevail and premature recommendations to public and patients be discouraged.

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Role of lipids in normal metabolism

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LIPIDS ARE NORMALLY CONSUMED in the diet, digested, absorbed, distributed and utilised in the mammalian body; most of them may also be derived by biosynthesis from non-lipid sources. There would appear to be differences in lipid metabolism in different animals, some of which may be significant. The object of this communication is to review briefly the main part that lipids play in normal metabolism, with particular reference to man. To understand the 'normal' in human studies it is also necessary to consider the 'abnormal', since this provides a situation comparable to experimental studies in animals.

DIETARY FAT

Some animals, such as guinea pigs, eat little fat in their natural diet and the results of studies involving feeding large amounts of lipids to such animals must be treated with caution. The rat, dog and cat, on the other hand, consume quantities more comparable with man; experimental studies on fat absorption in the rat, however, have sometimes involved grossly excessive amounts of fat—equivalent to doses of 10 g/kg body weight or more—although in recent years it has become the usual practice to use more moderate quantities. There is, however, a difference between the commonly used experimental situation in animals and man and that prevailing under more normal circumstances. In the former, fat alone is often fed, whereas in normal feeding the fat is accompanied by considerable amounts of other food materials. The presence of these other foods may be significant.

The normal intake of fat in man varies widely. It may be extremely high for people doing heavy physical work in cold climates—perhaps as much as 400 g a day. There are some indications from animal studies that fat in the diet helps to increase work capacity. It is not certain that this also applies to man. It has been shown, however, that human subjects maintain efficiency more effectively when exposed to cold if they are consuming a high-fat diet. Fat intake is often extremely low for poor people living in tropical or subtropical climates. It may be less than 15 g a day. The average amount of fat consumed by an adult man on a Western diet is 100–150 g a day. The question of the optimal level of fat in the diet is complex. Much depends upon the nature of the fats consumed and the distribution in them of other lipid-soluble dietary nutrients. The accepted level is usually determined by the food habits of the community. It has been suggested that fat should provide at least 25% and that essential fatty acids should account for at least 1% of the total calories. Considerably greater amounts of fat (more than 40% of total calories) may, however, be consumed on a mixed Western-type diet. The clearer definition of the optimum levels of fat intake is a matter of some importance.

The types of fat eaten also vary widely. Many vegetable oils contain relatively large amounts of polyunsaturated fatty acids, such as linoleic acid, and tocopherols. Animal fats, on the other hand, may contain more saturated fatty acids and have a relatively low tocopherol content. This is especially so in the fats of ruminant animals, since the unsaturated fatty acids in the animal's diet are extensively modified in the rumen. The fat of nonruminant animals, such as the pig, may contain considerable amounts of linoleic acid, if they are being fed on a diet that is a good source of this fatty acid. Milk fats from animals that mainly synthesise the fat from 2-carbon fragments may contain significant amounts of short chain fatty acids. These are present in cow's milk and its products. It is obvious that the fatty acid pattern of the diet may vary widely according to the amount and type of animal food consumed and the proportion of vegetable oils. It has been suggested that processing operations, such as hydrogenation, might adversely affect the proportions of saturated and unsaturated fatty acids. While it is true that extensive hydrogenation of oils might do this, the extent of hydrogenation commonly used for dietary fats has little significance. Margarine is a better source of polyunsaturated fatty acids than butter. It has also been suggested that the treatment of flour with oxidising agents, such as chlorine dioxide, destroys the unsaturated fatty acids. This has been shown not to be so. Normal levels of chlorine dioxide treatment, which only have a mild oxidising action, do not significantly alter the linoleic acid content of flour. However, this and other forms of flour treatment, including aeration and natural ageing, cause a decrease of tocopherols. The milling of the flour has removed the greater part of the α-tocopherol and about 60% of that left is ϵ-tocopherol of low biopotency. The total tocopherol content of flour is of the same order as that of animal fats, in which the active

α-tocopherol predominates. The reduction of flour tocopherols by maturing processes cannot be regarded as nutritionally significant, in relation to man.

Many fats undergo deteriorative changes and become rancid. Animal fats commonly show hydrolytic rancidity. This gives strong off-flavours and, so far as present evidence goes, appears to be relatively harmless. Vegetable fats, especially those containing a large amount of polyunsaturated fatty acids, tend to oxidise. Oxidative rancidity leads to the formation of peroxides, polymers and secondary oxidation products. This type of rancid fat has been shown to have toxic effects; it has been suggested as a possible factor in the induction of tropical sprue in Hong Kong. The use of vegetable oils for deep frying should be carefully controlled.

Nutritional advantages have been claimed for various fats, especially for butter against margarine, or for animal rather than vegetable fats. Many different indices, such as growth rate in normal, premature, or undernourished rats, and in calves, have been used, and investigations have also been carried out in children. These various studies indicate that there are no fundamental differences in the general nutritional value of ordinary dietary fats derived from animal or vegetable sources. It is clear, however, from the points already made that the way in which the fats are handled in their preparation as food materials should be given careful consideration. Apart from general nutritional value, the proportion of saturated and polyunsaturated fatty acids may be important in relation to blood cholesterol levels, atheroma and ischaemic heart disease. A diet chosen to contain an adequate supply of suitable polyunsaturated fatty acids appears to maintain a relatively low blood cholesterol level on a long term basis. Whether this will materially affect the incidence of ischaemic heart disease remains to be seen. The fact than an increased intake of polyunsaturated fatty acids will lower the blood cholesterol does not necessarily mean that the diet previously consumed was deficient in polyunsaturated fatty acids. The effect may be pharmacological rather than physiological. Most of the analyses that have been made on tissue lipids of subjects with atherosclerosis or ischaemic heart disease have not revealed any obvious shortage of polyunsaturated fatty acids. These problems are more fully discussed by Dr. Bronte-Stewart.

The nature of the dietary fat may also affect digestion and assimilation. It has been known for many years that the higher melting point fatty acids or glycerides are poorly absorbed unless they are provided with an adequate liquid oil vehicle. The fat in the mixed diet commonly taken by man is over 95 % absorbed, which would suggest that the proportions are normally adequate. In the case of ingestion of excessive amounts of a highly saturated fat of high melting point, less complete absorption may be observed. Some fatty acids are better absorbed than others. Recent studies suggest that erucic acid is not as well absorbed as oleic acid; the poor absorption of triricinolein is well known, although ricinoleic acid is well absorbed. Addition of large amounts of cholesterol may interfere with absorption of fats; this does not arise under ordinary circumstances. Recent studies by Fernandes, van de Kamer and Weijers suggest that completeness of absorption is dependent upon chain length and this becomes evident in children with defective absorption. The presence of one particular fatty acid may also affect the absorption of another. This raises the further question—the importance of the position of the fatty acid in the glyceride. It is possible that this may have a bearing on the effects of fat in the body.

PREPARATION OF LIPIDS FOR ABSORPTION

Having considered some of the main features of the dietary fats, we must now turn our attention to their digestion and absorption.

Hydrolysis. An appreciable amount of lipolysis of glycerides may occur in the stomach. The extent to which this is due to gastric lipase or regurgitation of pancreatic lipase is not clear. The main lipolytic activity would appear to be dependent upon the pancreatic lipase. If this were not so, it would be difficult to explain the marked effect of pancreatic lipase deficiency on fat absorption. Absence of pancreatic lipase usually results in reduction of fat absorption by about half.

The formation of fatty acids and lower glycerides by the action of pancreatic lipase has been extensively studied. The rate of liberation of fatty acids from glycerides during pancreatic lipolysis depends upon their position in the glyceride, the fatty acids in the 1 and 3 positions being most rapidly released. In the dynamic situation that prevails in the intestinal lumen, this differential rate of liberation may be important.

The extent of hydrolysis has been the subject of much discussion. Hydrolysis need not be complete in the intestinal lumen for absorption of fat to occur. There are many indications that hydrolysis may be less than 50 % under normal circumstances. The rate of hydrolysis is dependent upon the amount of enzyme in relation to substrate. When very little fat is used, the amount of hydrolysis may be almost complete. If the amount of fat ingested is greatly increased, there is no evidence of a corresponding increase in the amount of lipase made available, absorption, however, remains at over 95 %.

Emulsification. There is no doubt that fine emulsification of fats occurs in the small intestinal lumen. This can be readily demonstrated in man by intubation. The mechanism of emulsification appears to be the triple system fatty acid/lower glycerides/bile salts. Other substances may be involved, but, under normal circumstances, these 3 appear to be essential. Faulty emulsification can be demonstrated in patients with defective pancreatic lipase, since this prevents the formation of fatty acids and lower glycerides; it is also faulty in bile salt deficiency. Emulsification is re-established by the addition of the appropriate missing factor.

Hofmann has recently reported an elegant demonstration of micelle formation with the triple complex, fatty acids, monoglyceride and bile salts. It is possible

that the hydrophobic central portion of these micelles may be able to accommodate water-insoluble, lipid-soluble molecules. The concept that these micelles are the starting point of a particulate system that can be absorbed would be compatible with our earlier studies on the nature of the intraluminar emulsifying system. It may be of interest that mono-olein appeared to be specially effective for micelle formation.

What is the significance of fine emulsification? For many years it was regarded simply as a means of obtaining more complete hydrolysis. Studies with the electron microscope, however, have revealed the microvillous structure of the brush border of the small intestinal absorbing cells. Fatty material is not seen within the microvilli, but only in the spaces between them. There is a critical size factor for entrance to these spaces. Large fat particles cannot penetrate through the brush border and remain in the lumen; fine particles 0.1 μ in diameter, and possibly slightly larger, can penetrate readily to the bottom of the intermicrovillous crypts. The original estimate of critical particle size for fat absorption was less than 0.5 μ. Current measurements show that particles of 0.1 μ can be seen in the intermicrovillous spaces. The critical diameter is probably between 0.1 and 0.5μ.

The significance of fine emulsification to fat absorption is clearly demonstrated by studies in patients with pancreatic enzyme deficiency. Patients can be selected that have pancreatic lipase deficiency, but normal upper intestinal function, as shown by absorption rate tests with water-soluble index substances, by radiography and by biopsy. Emulsification of fat is defective. If unemulsified glyceride fat is administered intraduodenally in such a patient, no alimentary hyperlipaemia is evident. If, however, the same amount of fat is given finely emulsified, a normal alimentary hyperlipaemia occurs.

Faecal fat. The faecal fat in a normal individual is less than 5 g a day on an intake of about 100 g. It can be shown to be more saturated than the dietary fat. Recent studies by James, Kellock & Webb and by my colleagues, Sammans & Wiggs indicate that it contains an exceptionally high proportion of stearic acid and also fatty acids not present in the diet, such as oleic acid isomers and hydroxy stearic acid. It is to be expected that unabsorbed fat remaining in the large bowel for 12 hours or more in the presence of the intestinal flora might undergo certain modifications. This would appear to be the reason for the presence of hydroxystearic acid. It might also explain the oleic acid isomers and high proportion of stearic acid, but the present limited evidence does not support this. The intestinal flora are also responsible for changes in bile pigments, cholesterol and other substances in the stools. They play an important role in bile salt metabolism as pointed out by Professor Bergström. Studies with labelled fats and a simultaneous fat balance indicate that the fat in the normal stools consists of a mixture derived from several sources. Only about 50% at the most is derived from unabsorbed dietary fat. The faecal fat is mainly present as fatty acid. Even in the absence of pancreatic lipase, hydrolysis of faecal fats is usually greater than 80%.

If fat absorption is incomplete for any reason, the unabsorbed fatty material is passed in the stools. It is remarkable that satisfactory methods for determining the origin of this excessive fat in each individual patient are not yet available. The fat might be derived from unabsorbed fat, unchanged or modified, or it might come from other sources by synthesis or excretion. Changes in the fat load in the diet raise difficulties, since other dietary changes have to be made. The use of labelled fatty acids becomes complicated by the possible differences in the rate of absorption of different fatty acids and the possible significance of the position of the fatty acid in the glyceride. Recent studies by Sammans have made use of the conversion of erucic to behenic acid as a means of studying intraluminar hydrogenation. The combination of these various methods with vapour phase chromatographic studies may lead to a more satisfactory understanding of these problems in the near future.

The classical steatorrhoeic stools are fluid or semi-fluid, bulky, pale, offensive and fatty. They commonly contain an excessive amount of both long-chain and short-chain fatty acids and nitrogen. The amount of pigment is normal, but it is paler in colour. Many of these changes are due to increased bacterial activity in the lower bowel. This is caused by the larger amount of food material available as a result of delayed or ineffective absorption. The strongly reducing fermentative flora account for the presence of short-chain fatty acids derived from carbohydrates, for some of the physical characteristics, such as fluidity and increased bulk, and for the pallor of the pigments. As already indicated, the flora may also cause hydrogenation of unsaturated long-chain fatty acids, with the formation of stearic acid and related compounds. In the majority of cases the long-chain faecal fat appears to be derived mainly from unabsorbed dietary fat, but, in a few cases, especially in sprue patients with persistent steatorrhoea, the fat seems to be derived from some non-dietary source. This form of steatorrhoea responds dramatically to treatment with antibacterial agents. The reason for this effect remains obscure. It may also be noted that the fatty acids in the stools may affect the intestinal flora.

It should perhaps be emphasised that the nature of induced changes in the intestinal flora should be carefully studied and that the possible significance of yeasts should not be forgotten. The mere fact that antibacterial agents have been given does not mean that significant floral changes have occurred. The flora may be resistant to the antibacterial agents used and, in any case, adaptation quickly takes place. Krondl has recently reported that neomycin and tetracyclines inhibit pancreatic lipase. This may account for an observation we have made recently that the administration of neomycin aggravates steatorrhoea.

Passage of excessive amounts of fat may lead to in-

creased loss of calcium and magnesium. The loss may be sufficiently severe to cause tetany, secondary hyperparathyroidism and bone changes. The calcium soaps may appear in the faece as fine crystalloids which give a watered silk appearance to the faecal extract.

Other effects of defective fat absorption may be considered. In many patients with steatorrhoea, there is severe loss of weight and disappearance of fat depots. This is particularly seen in gluten-induced enteropathy and tropical sprue. With appropriate treatment, body weight and fat fold thickness return to normal. These effects are not, however, due to the fat absorption defect, per se, but to the general calorie deficit resulting from anorexia. This is borne out by the fact that many patients with pancreatic enzyme deficiency, in which the fat absorption defect may be much more severe, show little change in body weight or fat fold thickness; such patients have no anorexia and may eat sufficient to cover the absorptive defect. Patients with steatorrhoea often show low phospholipid, cholesterol, vitamin A and tocopherol levels in the blood. This again is rather surprising in patients in whom the fat absorption defect may be quite small; 70–80% of the ingested fat is often absorbed. The explanation must lie either in the overall reduction of intake due to anorexia, the effects of regional interference with absorption, or faulty transport; more than one of these factors may be operative. It is well recognised that different substances are absorbed from different parts of the small intestine. Enteropathies may affect different parts of the small intestine and cause a dissociation between the overall absorption of fat and various fat-soluble substances.

One further effect of fat in the diet should be mentioned. Fat appears to be closely associated with several gastrointestinal hormones. Thus, it may be involved in the control of gastric emptying time, in the emptying of the gallbladder and perhaps in other coordinating mechanisms. It is generally thought that fat improves the palatability of the diet; it seems likely that it may also play a part in determining the timing of the digestive phases and the acceptable interval between meals.

Intestinal cell and lipids. As already mentioned, fatty material may enter the intestinal cell from the bottom of the intermicrovillous crypts. Particulate fat can only reach this situation if it is finely emulsified to a particle size of the order of 0.1 μ. Some glycerides, such as triricinolein, are not readily emulsified and absorption is consequently defective. Paraffins alone are also poorly absorbed, but if provided with an effective emulsifying system, absorption can occur. Interference with the dispersibility of fat, for example, by polymerisation, may also interfere with its absorption.

Fatty acids in water solution will presumably enter the cell with other water-soluble substances. Whether the pathway is different from that used by particulate material remains to be established. Water-soluble materials are well absorbed in pancreatic lipase deficiency, but glyceride fats are not. Shorter chain fatty acids can be readily solubilised in water and this becomes more difficult with increasing chain length. The presence of bile salts or other substances may, however, assist. Cholesterol esters are hydrolysed and the cholesterol is absorbed. Phospholipids may be broken down, but active phospholipase cannot always be demonstrated in the intestinal lumen; it appears that these molecules may pass into the cell intact. Active lecithinases are present in the intestinal mucous membrane extracts and are probably located in the intestinal absorbing cells.

Thus, under normal circumstances the cell receives fat containing longer chain fatty acids and other fat-soluble materials in finely dispersed particulate form. Pinocytosis of particulate fat by intestinal cells has been demonstrated. It is not known whether this accounts for the whole or only part of the absorption of glyceride fats. Water also flows through the cell in large quantities and in it may be dissolved a wide range of other substances, including certain fatty acids and glycerol.

In a short time fatty material begins to accumulate in the cell in particulate form. The emulsion does not appear to be stable and the particles coalesce and increase in size. It is difficult at the present time to be sure that this is not an artefact brought about by feeding too much fat and not enough other food materials, which may be necessary for the most effective functioning of the cell. These fat particles accumulate in the spaces of the endoplasmic reticulum. They pass through the cell and are eventually discharged from the side of the cell into the intercellular spaces. From here they find their way into the corium of the villus. These main features of the cellular phase of fat absorption have been demonstrated in animal studies and confirmed from biopsy studies in man.

Examination of the fat particles in the intestinal lumen and in the intestinal lymph show that certain definite changes have occurred. The main points are the disappearance of short-chain fatty acids (less than 10 carbon chain length), the resynthesis of triglycerides and the increase in the amount of phospholipid present, mainly as phosphatidyl choline. Studies on the intestinal mucous membrane reveal the presence of enzymes that can readily resynthesise triglycerides. Dawson and Johnson, working independently, have recently demonstrated that the synthetic pathway for glycerides described by Kennedy can be utilised by the small intestinal mucosa. Phosphatidic acid is an intermediate in this biosynthesis. My colleagues, Hübscher and Clarke, have shown that the enzymes involved in this system are present in mitochondrial preparations from small intestinal cells. They have further demonstrated that di- and triglycerides can be formed from monoglyceride in these mitochondrial preparations without the intervention of phosphatidic acid. This alternative pathway is not a simple reversal of lipase action. It is interesting that mono-olein was found to be particularly active in triglyceride resynthesis. The short-chain fatty acids and free glycerol liberated by lipolysis pass from the intestinal cell into the portal blood and are not involved in glyceride resynthesis in the intestinal cell. The fatty material in the intestinal lymph contains 6–10% of phosphatidyl choline and this con-

tains fatty acids derived from dietary triglycerides. The exact site of these metabolic changes in the intestinal mucosa is still unknown.

The fat particle has also changed its physical characteristics. It is now compatible with plasma proteins and has a phospholipid/globulin stabilising film which is readily attacked by D-lecithinase from Cl. Welchii toxin. It seems probable that this association with lipoprotein occurs in the intestinal cell, but final proof of this has not been obtained. It is possible that the close association with protein only occurs when the particle reaches the lymph. Under certain circumstances the particle may have a different structure and it may then behave differently in the body. For example, in choline deficiency in the rat, it is possible to obtain fat particles in the chyle that are resistant to D-lecithinase and are more slowly cleared than normal by heparin 'clearing factor'. Whether similar changes happen in man remains to be established; we have observed peculiar particles of this type in the chyle in 1 patient, but, unfortunately, we were unable to establish the cause. Some years ago we demonstrated that the addition of choline to glyceride increased the amount of fat absorbed in three hours by 50%. This was an immediate effect similar to that described by Dr. Hartroft. It seems likely that other dietary components may play an important part in the effective handling of fat in the intestinal cell and this, in turn, may have later repercussions on lipid metabolism. The presence of upper intestinal juices has been shown to play some part in glyceride resynthesis in the rat small intestine. Recent work by Dawson suggests that the presence of conjugated bile salts may be significant. It is conceivable that the supply of fats to the intestinal cell, which are required for the formation of phosphatides, may be of some importance in connection with the absorption of other non-fatty substances. Further studies on these inter-relationships are urgently needed.

Recent developments in gastroenterology have made it possible to study the small intestinal mucous membrane in human subjects by peroral biopsy. Severe damage to the mucous membrane and small intestinal cells can be demonstrated in many patients with steatorrhoea, especially those with gluten-induced enteropathy and tropical sprue. It is difficult, however, to find any close correlation between the cellular damage and the overall absorptive defect. This is, perhaps, not surprising, since the damage may be relatively localised, whereas overall absorption is a measure of the functional efficiency of the small intestine as a whole. Attempts have been made to interfere with the specific mechanisms concerned in the handling of fat by the small intestinal cells. Many years ago moniodoacetic acid was used; its interfering effect, however, was largely due to changes in motility and the fat appeared to pass readily through the intestinal cells. More recently the effects of folic acid deficiency have been studied; present evidence indicates that reduction in the number of absorbing cells, rather than any specific defect in the fat absorption mechanism, may account for any effects observed.

Pathways of absorption. Under normal circumstances, the visible particulate fat that leaves the intestinal cell passes selectively into the central lacteal. The classical method of demonstrating lymphatic vessels anywhere in the body is to inject particulate dyes. This particulate matter enters lymphatics in preference to blood capillaries. This effect is seen in the intestine. The water-soluble materials, and amongst them one must include water-solubilised fatty acids, pass mainly in the portal blood. Presumably some will also pass into the lymphatic system, but since the volume of the portal blood flow is extremely large as compared with lymph flow, the distribution of water-soluble substances appears to be primarily portal.

Blockage of the lymphatic pathway interferes with fat absorption. This may be seen in patients with exudative enteropathy as described by Gordon. In such patients, the lacteals and submucous lymphatics may be greatly distended, due to lymphatic obstruction; protein is passed into the intestinal lumen and although it may be digested and reabsorbed, this results in such a rapid turnover of the plasma protein that synthesis cannot keep pace with the rate of loss; consequently, hypoproteinaemia and oedema develop. If the hypoproteinaemia and oedema can be brought under control, the absorptive deficiencies may cause little difficulty. Absorption by the portal pathway is not affected unless there is generalised small intestinal failure. Interference with fat absorption due to blockage of the lymphatic pathway is sometimes inferred on the basis of the radiographic demonstration calcified mesenteric glands. However, such changes may be present without any apparent obstruction. It is also of interest that in two of our cases on which such a report was made, the opacities were found to be due to pancreatic stones and absence of pancreatic enzymes was the cause of the malabsorption.

Normal response to a fatty meal. After a normal meal containing fat there is a characteristic alimentary hyperlipaemia. This can be studied by serial analyses or by the chylomicrograph technique. The peak of the hyperlipaemia usually occurs in 2½–3 hours and normal blood fat levels are regained in 5–6 hours, provided that excessive amounts of fat are not used. The fat in the blood is in at least 3 forms: visible particulate fat, the chylomicrons, invisible particulate fatty material and unesterified fatty acid, commonly bound to albumin. The main increase in the alimentary hyperlipaemia is in the chylomicron fraction; the main chemical changes observed are an increase in glyceride fat, with some concomitant increase of phospholipid.

The fat particles are removed from the blood in a few hours. The rate of removal in the rat can be delayed by the administration of protamine. In human subjects the rate of removal may be delayed in some people. This is best shown in patients with essential hyperlipaemia. The rate of removal in such patients is so slow that the glyceride fat accumulates in the blood and may reach a level of 10 g/100 ml or more. If a low fat diet is taken, the blood fat gradually falls towards more normal

levels. The high level of glyceride fat does not appear to cause any short-term difficulties; the condition is usually discovered accidentally when blood is drawn for some other purpose and found to be grossly lactescent. Whether the condition predisposes to atheroma is not certain.

Removal of absorbed fat. The fatty material in the blood after a fatty meal is removed into the cells. Deposition in the adipose tissue cells and liver has been demonstrated. The proportions that reach these various destinations are not certain and may vary under different metabolic conditions. While the lipoprotein lipase liberated by heparin administration may play a part in the clearing of a normal alimentary lipaemia, this is not fully established. The liberation of the enzyme by particulate fat only occurs in some species of animals—indeed, it may be more marked in some strains than others. The enzyme may still be an important factor in clearing, even if it is not liberated into the blood stream, but acts at the cell surface, or within the cell. It is possible, however, to demonstrate the penetration of lipid into cells, especially liver cells, under conditions that appear to preclude intervention by the lipoprotein lipase. Present evidence suggests that, as in the case of the intestine, there may be at least 2 pathways for absorption—one dependent upon hydrolysis, in which the lipoprotein lipase is likely to play an important part, and the other independent of hydrolysis. Much more work is needed before any final conclusions can be reached on the mechanism involved in the clearing of alimentary lipaemia in man.

Utilisation of dietary lipids. It is apparent that dietary fat may be deposited in fat depots where it forms a valuable store of energy. These cells may also accumulate fat derived from carbohydrate by biosynthesis. These depots are turned over and are consequently reduced by ingestion of a calorie-deficient diet.

The nature of the fat in the fat depots tends to have some resemblance to the fat ingested in animals that consume large amounts of fat. The smaller the fat intake, the less does the depot fat resemble the dietary fat, since dilution with biosynthetic fat is correspondingly greater. In ruminant animals the dietary fat undergoes considerable modification in the rumen and the depot fats are consequently different from the fat eaten; the total amount of fat ingested by these animals may also be relatively small and much fat may be formed from other sources. The modifications in the dietary fat in man have already been discussed; further modification of the fatty acid pattern will arise from selective esterification of fatty acids—an aspect of fat metabolism requiring further study.

Another important use of glyceride fats in some species is their excretion in milk during lactation; some animals, especially ruminants, form milk fat in the mammary gland mainly from 2-carbon fragments, or other small molecules. Glyceride fat is also useful as a space-filling, mobilisable material; as such, it is found in long bones as the yellow bone marrow.

The main uses of lipids are in tissue structure and energy production. Cholesterol and phosphatides are particularly concerned in the former. In association with proteins they form cell membranes and intracellular organelles. They play an important part in the nervous system, since lipids are essential components of myelin. The lipids involved in cell structure are not merely static components; there is ample evidence that they play an important part in the functional activity of the structures concerned. Not only are these lipoproteins important in cells; they are also components of body fluids, such as plasma, where they may be regarded as having essentially a structural role.

The main energy-producing lipids are the glycerides. The fatty acids are metabolised by many cells; they may perhaps be regarded as the preferential fuel for cardiac muscle cells, but they are not significantly utilised by brain cells. The body as a whole makes a continuing use of fatty acids, as shown by the normal respiratory quotient of less than 1.0. When fat is absorbed a proportion of the fatty material passing into the body is soon oxidised; this can be readily demonstrated using labelled fatty acids. The rate of oxidation of the different long-chain fatty acids does not vary greatly. The final stages of fatty acid oxidation are dependent upon normal active glucose metabolism.

Another important aspect of lipids is the biological activity of certain lipid molecules. This is particularly the case with sterols. Biologically active sterols are produced in various cells, such as those of the adrenal cortex and the gonads. They play a vital part in the normal endocrinological control of body functions. Phosphatides may also have some important biological properties.

In conclusion, it may be said that many of the lipids found in the body can be synthesised from nonlipid components. Thus, the majority of fatty acids, glycerides, cholesterol and many phosphatides can be biosynthesised. Nevertheless, there are some lipid molecules that must be taken in the diet. The essential fatty acids, the linoleic acid family, fall into this category in many species. Choline, or transmethylating agents that will assist in the biosynthesis of choline, vitamin A or B carotines and tocopherols are other examples of dietary essentials of importance in lipid metabolism. However, apart from the substances that cannot be formed in the body, it is unlikely that the body could maintain a normal economy if all the lipids had to be synthesised from non-lipid precursors. There is some evidence that the inclusion of fat in the diet is in itself beneficial, both as regards growth and fertility. Since the nature of the fatty acids contained in fats and the presence of phosphatides and other lipid molecules may have considerable repercussions on general body metabolism, more detailed study of the nutritional importance of dietary lipids is needed.

REFERENCES

The older references will be found in standard works on lipid metabolism, such as "The Lipids" by H. J. Deuel, Jr. Most of the recent studies have been discussed at meetings and are now in process of publication.

Endocrines in lipid metabolism

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LIPID METABOLISM embraces the absorption, transformation, storage and disposal of all lipids by living cells. In this paper it will be necessary to restrict the discussion to one lipid—namely cholesterol. The particular facets of this lipid which will be dealt with include factors affecting cholesterol synthesis and plasma storage in 2 species, the rat and the human.

The complex mechanism by which cholesterol is synthesised by cells has been reviewed recently (6, 7, 15). On a sterol-free but nutritionally adequate diet, the concentration of cholesterol in the tissues and tissue fluids of an adult mammal is surprisingly constant. The precise mechanism by which the biosynthetic rate is delicately balanced to cellular requirements is still obscure. Opinions on the nature of the intracellular control of biochemical processes appear to be divided. On the one hand there are the studies which emphasise the importance of the 'primitive control mechanisms'; equilibrium is then explained by autoregulation processes within the cell by which the concentration of certain 'key substrates' dictates reaction rates (25, 26). On the other hand there have been many attempts to implicate hormonal influences in the control of lipid metabolism (11, 19, 40).

There can be little doubt that in multicellular organisms both mechanisms are operative, one perhaps fulfilling the role of a 'fine control' on the other more basic regulator.

Hypercholesterolaemia occurs spontaneously in certain animals and humans, sometimes in association with certain endocrine dysfunctions, while in other instances the aetiology is obscure. We decided to investigate certain aspects of the endocrine control of the plasma cholesterol employing standard endocrinological and biochemical procedures. The data to be presented in this paper will be restricted in two ways. Firstly, the experiments will be limited to comparing and contrasting experimental findings in the rat and in man. Secondly, only 3 endocrine glands or secretions of these glands will be considered, namely the gonads, the thyroid and the adrenal cortex.

MATERIALS AND METHODS

Animals

These studies were made on male and female adult rats of an inbred Wistar strain maintained in this colony for many years. The animals were free from obvious disease, and they grew and bred normally. They were maintained at an environmental temperature of 25°C and had access to water ad lib. These rats were fed a basic diet consisting of 70% wholemeal flour, 25% skimmed milk powder and 5% brewer's yeast.

Castration, thyroidectomy, and adrenalectomy were performed under ether anaesthesia using standard operative procedures. Thyroidectomy was also achieved in certain cases by total ablation of the gland by radioactive iodine.

Humans

The plasma lipid studies were all confined to human male volunteers who had clinical evidence of ischaemic heart disease. The subjects were all outpatients and no attempt was made to control their diet except to advise them to refrain from making any alteration to their eating habits. Many of the subjects were survivors of a myocardial infarction, but all observations reported here were made at least 3 months after the acute phase of the illness.

Plasma cholesterol was determined by the procedure of Sperry and Webb (39). 'Half-life' of plasma cholesterol was determined in animals by the intraperitoneal injection of 30 μC sodium acetate-1-C^{14} in 100 μM. Thereafter, at intervals, blood samples were taken from a tail vein for cholesterol and C^{14} determinations. The procedure has been described by Boyd and Mawer (10). From these data by a semi-log. plot the biological half-life of the plasma cholesterol can be obtained. The analytical procedure involves the rupture of lipoprotein and cholesterol ester linkages with the final treatment of the plasma cholesterol as if it were a homogeneous entity. This procedure gives a result which is the average half-life of a vast number of different cholesterol complexes present in plasma. Investigations are in progress in many centres, including our own, in an attempt to refine these methods and obtain complete fractionation of the cholesteryl esters of plasma. If and when these efforts are successful, the determination of rates of disappearance of the individual plasma cholesteryl esters may be possible and thus a better approximation to the physiological situation may emerge. In the meantime, it is necessary to treat the plasma cholesterol as if it were a homogeneous unit,

acknowledging that subsequent reassessment will be necessary.

As it was intended to pursue endocrinological studies on sterol metabolism over relatively long observation periods, preliminary experiments were necessary to establish the effects of sex differences and age trends on cholesterol metabolism in the rat and in man.

SEX DIFFERENCES AND AGE TRENDS

Rats

Male and female rats (12 per group) were housed in separate cages, fed the stock diet and treated exactly as detailed previously. At fixed intervals the animals were bled from the tail (0.4–0.5 ml blood) and the plasma cholesterol levels determined. The results are shown in table 1. The higher plasma cholesterol level found in female rats of this colony fed on the stock diet is highly significant ($P < .001$). These results are in general agreement with the findings of Fillios, Kaplan, Martin and Stare (18). These studies illustrate that, in the rat, the plasma cholesterol of the female is higher than that which prevails in the male. The plasma cholesterol also exhibits in both sexes a fall followed by a general upward rise with advancing age. These findings are in general agreement with the observations of Bargeton, Krumm-Heller and Tricaud (3).

Humans

The plasma cholesterol levels of normal male and female subjects (20 subjects per group) between 21 and 40 years of age have been determined in a section of the population of Scotland and are shown in table 2. Thus, in the human, in the age range chosen, the plasma cholesterol sex difference is absent and the rise with advancing years is less well marked. The plasma cholesterol increment with age has been studied extensively.

GONADECTOMY IN MALE

Rats

Twenty-four young adult male rats were randomly separated into 2 groups; 1 group was subjected to castration, while the other was sham-operated. The animals were kept on the stock diet and bled from the tail at intervals for plasma cholesterol determinations. The results of these studies are shown in table 3, from which it can be seen that, in the male rat, castration is followed by a slight but significant rise in the plasma cholesterol.

Half-life of plasma cholesterol. Six animals from each of the previous groups (castrated and sham-castrated) were injected with sodium acetate-1-C^{14} as previously described and the semi-log. plot of the specific activity of the plasma cholesterol of the 2 groups is shown in figure 1. These results show that, after castration in the male rat, the apparent relative rate of plasma cholesterol synthesis is increased and the biological half-life of the plasma cholesterol is not significantly altered.

TABLE 1. *Effect of Age on Plasma Cholesterol Level of Rat*

Age, months.....	1	3	5	8	10	12
Males	78	61	66	54	55	69
Females	71	84	88	90	81	80

TABLE 2. *Effect of Age on Plasma Cholesterol Level of Human*

Age group.....	21–25	26–30	31–35	36–40
Males	187	183	193	198
Females	180	189	186	190

TABLE 3. *Effect of Castration on Plasma Cholesterol Level of Male Rat*

Time after operation, months.....	1	2	4	6	9	12
Sham-operated	66	61	68	74	69	60
Castrated	64	73	81	84	74	77

Humans

Furman and his co-workers (20, 21) in a study of eunuchs have shown that the plasma cholesterol concentration is lower than normal after orchidectomy. Up to the present no reports have appeared on the biological half-life of the plasma cholesterol in gonadectomised human males.

OVARIECTOMY IN FEMALE

Rats

Twenty-four young adult female rats were randomly separated into 2 groups; 1 group was subjected to bilateral ovariectomy, while the other was sham-operated. These animals were kept on the stock diet and bled from the tail at intervals for plasma cholesterol determinations. It can be seen from the results shown in table 4 that, in the female rat, ovariectomy is followed by a marked elevation of the the plasma cholesterol level. These results are in agreement with the observations of Fillios (17).

Half-life of plasma cholesterol. Six animals from each of the previous groups (sham-operated and ovariectomised) were injected with sodium acetate-1-C^{14} as previously described and the semi-log. plot of the average specific activity of the plasma cholesterol is shown in figure 2. These results show that ovariectomy in the female rat results in a diminished apparent relative rate of plasma cholesterol synthesis and an increased biological half-life of this component. These findings are in general agreement with the observations of Fillios, Kaplan, Martin and Stare (18).

Humans

The influence of the ovaries on the plasma cholesterol level in the human has been studied in groups of females before and after the menopause. Oliver and Boyd (32)

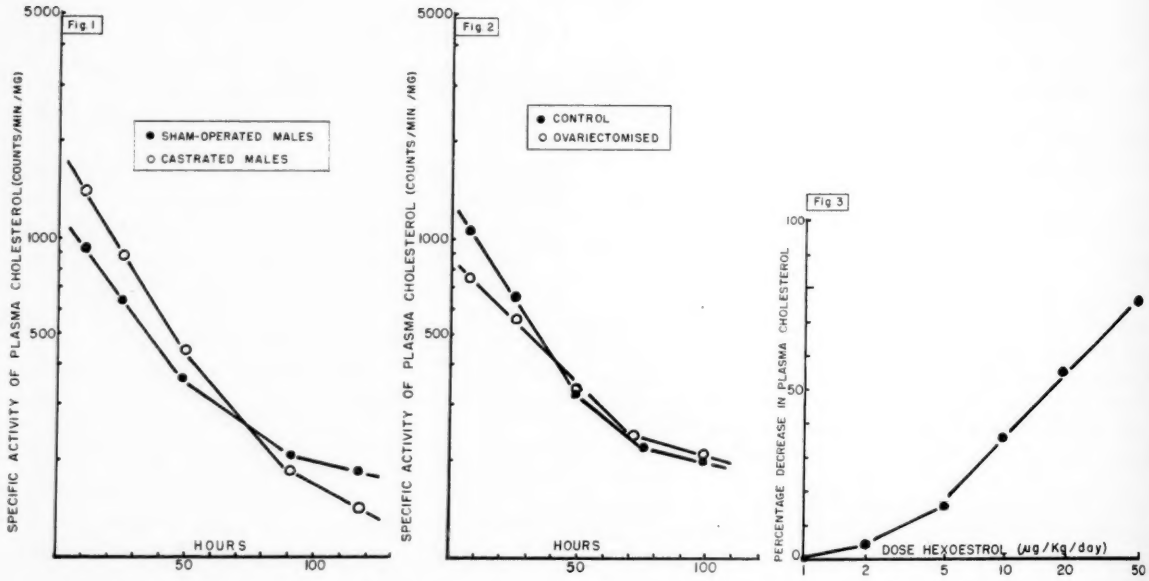

FIG. 1. Effect of castration on the biological half-life of plasma cholesterol in male rats.
FIG. 2. Effect of castration on the biological half-life of plasma cholesterol in female rats.
FIG. 3. A dose-response curve of the effect of hexoestrol on plasma cholesterol in male rats.

TABLE 4. *Effect of Ovariectomy on Plasma Cholesterol Level of Female Rat*

Time after operation, months....	1	3	5	6	8	10	12
Sham-operated	73	68	79	84	69	87	88
Ovariectomised	101	88	91	87	104	83	97

TABLE 5. *Effect of Hexoestrol on Plasma Cholesterol Level of Male Rat*

Time, days.....	0	1	3	5	7	10
Plasma cholesterol, mg %	83	70	53	42	38	41

showed that women over the age of 50 years had significantly higher plasma cholesterol levels than younger women, the increment being of the order of 30–40 mg %. This has been confirmed by Adlersberg *et al.* (2) and Robinson *et al.* (36). After bilateral ovariectomy there is a rise of the plasma cholesterol to the level observed in post-menopausal women (35).

EXOGENOUS SEX HORMONES

Rats

Oestrogens. The effects on cholesterol metabolism of a large number of oestrogenic substances—both naturally occurring and synthetic—have been studied in the rat. Levin (27) showed that stilboestrol depressed the plasma cholesterol of the rat. Hexoestrol, oestrone, and oestradiol were studied in the rat in this laboratory (9, 31). These studies have been extended in an attempt to obtain information on the mode of action of oestrogens in cholesterol metabolism.

Hexoestrol. The administration of 50 µg hexoestrol/100 g body wt. to 12 adult male rats for 10 days resulted in all the usual manifestations of the oestrogenised male. The plasma cholesterol of the group was followed throughout and is shown in table 5. Thus, this dose of hexoestrol exerts a potent depressant effect on the plasma cholesterol of the male rat.

In order to establish whether the hypocholesterolaemic response was related to the dose of hexoestrol, 6 groups, each of 6 adult male rats, were treated with doses of hexoestrol varying from 0–50 µg/100 g body wt. for 7 days. The plasma cholesterol was determined at the conclusion of the experiment and the percentage depression of the plasma cholesterol plotted against the log. of the dose of hexoestrol. The results of this experiment are shown in figure 3. Thus, within certain limits, the percentage plasma cholesterol depression is linearly related to the log. of the dose of hexoestrol. An almost identical relationship has been shown to exist for stilboestrol.

Half-life of plasma cholesterol. Six control adult male rats fed on the stock diet were injected with propylene glycol, while 6 similar animals were injected with 50 µg hexoestrol in propylene glycol/100 g body wt./day. After 7 days, the animals were injected with labelled acetate as before and the biological half-life of the plasma cholesterol determined. The results, shown in figure 4, demonstrate that the administration of these large doses of hexoestrol to the adult male rat results in a slight diminution of the apparent relative rate of plasma

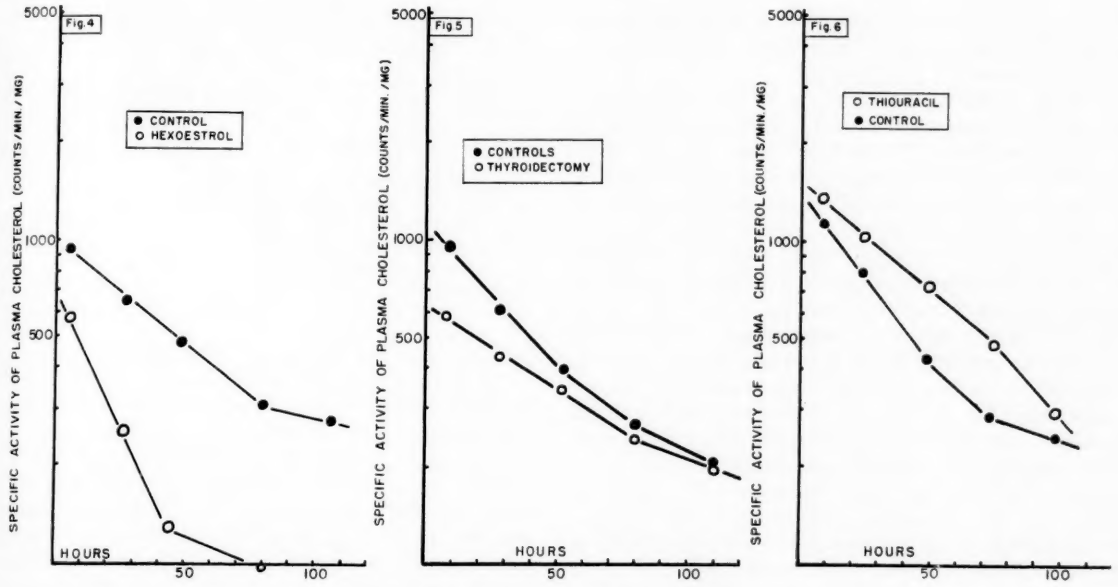

FIG. 4. Effect of hexoestrol on the biological half-life of plasma cholesterol in male rats.
FIG. 5. Effect of thyroidectomy on the biological half-life of plasma cholesterol in male rats.
FIG. 6. Effect of thiouracil on the biological half-life of plasma cholesterol in male rats.

cholesterol synthesis and a large reduction in the biological half-life of this molecule. Thus, the depression in the plasma cholesterol concentration is presumably due to an accelerated removal from plasma. Similar results have been obtained using stilboestrol. Oestrone, oestradiol, and oestriol when administered to the young adult male rat at a high dosage produce a depression of the plasma cholesterol similar to that described with hexoestrol. These hormones can also produce, at certain dosages, an increase in the apparent relative rate of plasma cholesterol synthesis, and decrease the biological half-life of the plasma cholesterol (Boyd, to be published).

Humans

The plasma cholesterol depressant effect of the administration of oestrogens to human male subjects was first described by Eilert (16). These observations have been confirmed and extended (4, 33, 37). In general, the administration to human males of any oestrogenic substance, whether natural or synthetic, at a dose level which ultimately proves to be 'oestrogenically active' results in a concomitant reduction in the plasma cholesterol level. Some results obtained in this laboratory are shown in table 6.

TABLE 6. *Effect of Various Oestrogens on Plasma Cholesterol Level of Male Human*

Oestrogen	Dose, mg/day	Plasma Cholesterol, % age depression
Ethinyloestradiol	0.20	21
Hexoestrol	60	10
Oestrone	50	10
Oestradiol	25	12
Oestriol	100	10

TABLE 7. *Effect of Thyroidectomy on Plasma Cholesterol Level of Male Rat*

Time, months	3	4	6	8	10	12
Control	69	76	60	58	63	66
Thyroidectomised	73	64	78	84	88	81

THYROIDECTOMY

Rats

Elimination of thyroid function is usually achieved by either surgical removal of the gland, ablation of the gland by radioactive iodine at high dosage, or by the administration of an antithyroid drug of the thiouracil type. Cholesterol metabolism in the rat appears to be influenced similarly by surgical removal of the gland or by radioiodine ablation, but thiouracil appears to exert an effect on cholesterol independent of its antithyroidal action (8).

Ablation of the thyroid of 12 young adult male rats was achieved by the intraperitoneal injection of 700 μC I^{131}/100 g body wt. The same number of litter mate male control animals were housed under identical conditions. Within 8 weeks the oxygen consumption rate and heart rate of the thyroidectomised rats were 60% of those of the controls. The plasma cholesterol of both groups was determined at intervals for a year. The results (table 7) show that, on a long-term basis, thyroid ablation in the rat produces a small but significant elevation of the plasma cholesterol level. By contrast, the administration

of a goitrogenic dose of thiouracil to male rats of the same colony produces a much more marked hypercholesterolaemia (8).

Half-life of plasma cholesterol. Six of the hypothyroid rats and 6 controls were selected 4 months after the start of the experiment. The animals were injected with labelled acetate and bled at intervals thereafter exactly as described previously. The log. of the specific activity of the plasma cholesterol plotted against time is shown in figure 5. Thus, in the hypothyroid state, the biological half-life of the plasma cholesterol is increased, while the apparent relative cholesterol synthesis rate is decreased. The decreased synthesis of cholesterol by hypothyroid animals was first reported by Karp and Stetten (24) and confirmed in humans by Gould and co-workers (23).

Thiouracil. Six young adult male rats were fed the stock diet containing 0.3% thiouracil for 3 weeks and then injected with labelled acetate as described previously. The results are shown in figure 6. The apparent relative plasma cholesterol synthesis rate and the biological half-life were both greater in these animals than in the controls (previous experiment). This increased synthesis in the presence of a prolonged half-life may account for the elevation of the plasma cholesterol observed in thiouracil-treated animals (5, 8).

Humans

Hypercholesterolaemia occurring in association with myxoedema was first reported by Mason, Hunt and Hurxthal (30) and there appears to be, in general, a very rough inverse relationship between the basal metabolic rate and the hypercholesterolaemia. Recently we have had the opportunity to study the effect of 'radioiodine-induced' reduction of thyroid function (for therapeutic reasons) in a group of 6 males who were initially euthyroid. The results of this study are shown in table 8.

EFFECT OF EXOGENOUS THYROID HORMONES

Rats

The effects of the administration to rats of thyroxine, triiodothyronine, and synthetic substances related to the thyroid hormones have been reviewed by Boyd and Oliver (12). The normocholesterolaemic rat rendered hyperthyroid does not exhibit a marked or reproducible hypocholesterolaemia. However, the production of a mild hypercholesterolaemia in this species yields an animal preparation which responds to thyroactive compounds by plasma cholesterol reduction.

When adult male rats are fed the stock diet described previously with 10% olive oil, 1% cholesterol, and 0.5% cholic acid, plasma cholesterol levels comparable to 'high human' levels are produced. The simultaneous administration of iodothyronines to these animals results in depression of the plasma cholesterol and this animal preparation has been used in this laboratory to study synthetic iodothyronines for hypocholesterolaemic activity (12). A representative selection of some of the substances which we have examined is shown in table 9, and it can be seen that all these thyroxine analogues have the ability to depress the plasma and liver cholesterol levels (12).

TABLE 8. *Effect of Partial Ablation of Thyroid on the Plasma Cholesterol Level of Male Humans*

Time after treatment, months...	0	1	3	4
Plasma cholesterol, mg %	168	177	203	240

TABLE 9. *Effect of Various Iodothyronines on Plasma Cholesterol Level of Male Rats*

Iodothyronine	Dose, mg/kg diet, to Produce a 50% Depression of Plasma Cholesterol in 14 Days
3,5,3′,5′-tetraiodo-L-thyronine (LT_4)	1.1
3,5,3′,5′-tetraiodo-D-thyronine (DT_4)	7
3,5,3′-triiodo-L-thyronine (LT_3)	0.30
3,5,3′-triiodo-D-thyronine (DT_3)	0.70
3,5-diiodo-L-thyronine (LT_2)	30
3,5-diiodo-D-thyronine (DT_2)	50

Half-life of plasma cholesterol. Euthyroid young adult male rats were fed the stock diet and selected groups of rats were given certain iodothyronines in the diet for a period of 14 days. The biological half-life of the plasma cholesterol of the control animals and the hyperthyroid animals is shown in figures 7–9.

These results show that the hyperthyroid state is accompanied by a reduction of the biological half-life of the plasma cholesterol and a varied response in the apparent relative plasma cholesterol synthesis rate.

Humans

We have studied the effects on the plasma cholesterol in the human of the administration of the naturally occurring thyroid hormones LT_4 and LT_3 and various synthetic iodothyronines including DT_4, DT_3, DT_2 and LT_2. A summary of our results (13) is presented in table 10. These results show that all the iodinated thyronines had the ability to reduce the plasma cholesterol in man. The optimum activity was found in the 3,5,3′-triiodo group and in all cases the natural L-isomer was more active than the D-isomer.

Half-life of plasma cholesterol. The incorporation of acetate into the plasma cholesterol has been studied in myxoedematous subjects by Gould (22) and Lipsky *et al.* (28). In both studies the percentage incorporation of isotope into plasma sterol was reduced in the hypothyroid state, and Gould (22) showed that L-thyroxine therapy increased the rate of synthesis. This author concluded that the rate of cholesterol synthesis in the myxoedematous state was about one-half to one-third of the normal rate.

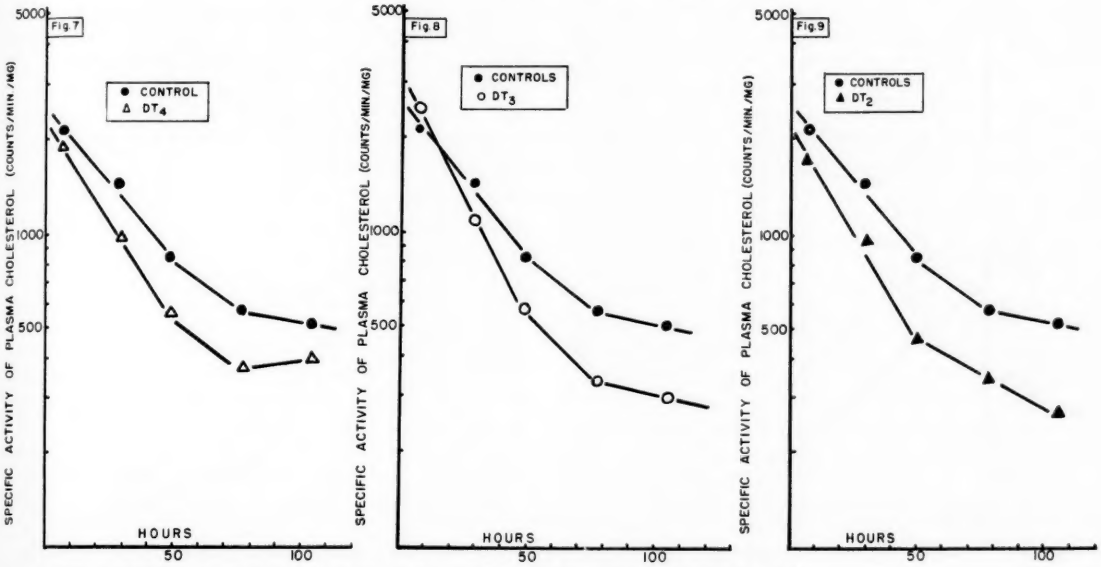

FIG. 7. Effect of DT_4 on the biological half-life of plasma cholesterol in male rats (10 mg/kg diet).
FIG. 8. Effect of DT_3 on the biological half-life of plasma cholesterol in male rats (1 mg/kg diet).
FIG. 9. Effect of DT_2 on the biological half-life of plasma cholesterol in male rats (100 mg/kg diet).

TABLE 10. *Effect of Various Iodothyronines on Plasma Cholesterol Level of Male Human*

Iodothyronine	No. of Subjects	Approx. Dose/Day, mg	Plasma Cholesterol, % age depression
LT_4	10	0.40	28
DT_4	12	15	26
LT_3	10	0.06	31
DT_3	12	0.50	26
LT_2	12	10	33
DT_2	20	200	24

ADRENALECTOMY

Rats

Adrenalectomy in the rat is a relatively simple procedure which results in the partial loss of 1 source of adrenaline and noradrenaline from the medulla, together with the loss of steroid secretions from the adrenal cortex. The amount of accessory viable adrenal tissue in the rat is very difficult to assess, but the majority of adrenalectomised young adult male rats survive for a considerable time when given no replacement therapy except 1 % sodium chloride to drink.

Both the adrenal catecholamines and the corticoids are known to influence lipid metabolism and consequently the interpretation of the mechanism underlying the results of adrenalectomy is fraught with considerable uncertainty.

One group of 10 young adult male rats was subjected to adrenalectomy and an identical group sham-operated. Both groups received 1 % sodium chloride to drink postoperatively. The animals were fed on the stock diet and bled at intervals for plasma cholesterol determinations. The results (table 11) indicate that there is a tendency for the plasma cholesterol to be lower after adrenalectomy in the adult male rat maintained on this stock diet.

TABLE 11. *Effect of Adrenalectomy on Plasma Cholesterol Level of Male Rat*

Time, days.....	12	18	30	40	60
Sham-operated	73	63	66	61	66
Adrenalectomised	63	58	51	60	50

Half-life of plasma cholesterol. The previous experiment was repeated exactly, except that 14 days after operation 6 adrenalectomised and 6 sham-operated rats were selected from each group. The animals were used to determine the biological half-life of the plasma cholesterol as described previously. The results of these observations are shown in figure 10. Thus, adrenalectomy in the adult male rat appears to produce a reduction in the apparent relative plasma cholesterol synthesis rate, while the analysis of the slopes of the curves suggests that, over the period studied, adrenalectomy did not significantly alter the biological half-life of the plasma cholesterol.

Humans

The effect of bilateral adrenalectomy on the plasma cholesterol has received very little attention. Individual values have been reported for the plasma cholesterol in subjects with adrenal insufficiency (29), but only short-term studies have been made on the plasma lipids following the second stage of adrenalectomy. This

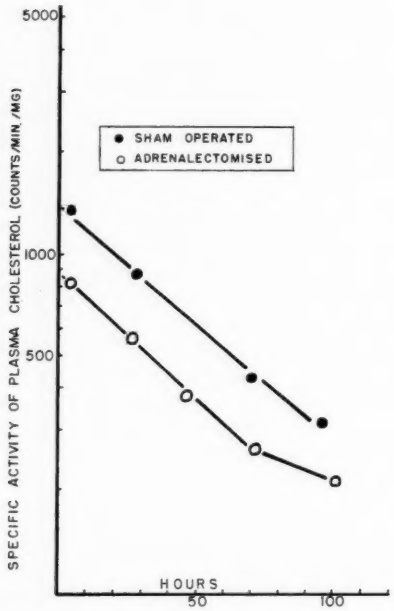

FIG. 10. Effect of adrenalectomy on the biological half-life o plasma cholesterol in male rats.

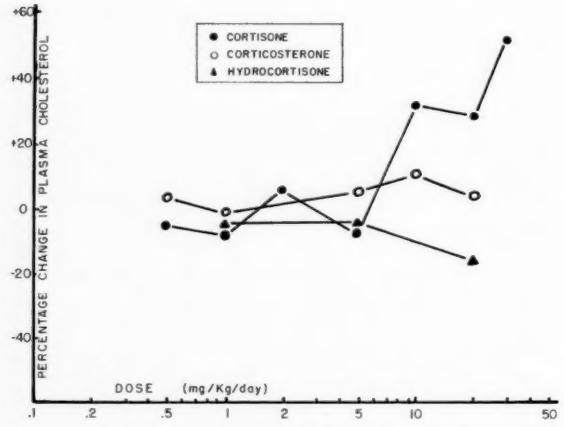

FIG. 11. A dose-response curve of the effect of adrenal cortical hormones on plasma cholesterol in male rats.

serious procedure is only carried out on pathological subjects and partial replacement therapy is usually instituted at once. This presents difficulties in the assessment of the degree of adrenal insufficiency in these patients.

EXOGENOUS ADRENAL CORTICAL HORMONES

Rats

The effect on the plasma cholesterol of the administration of adrenal cortical steroids has been studied in young adult male rats. The adrenal hormones which are known to be secreted by the rat are corticosterone and hydrocortisone. Corticosterone, cortisone, and hydrocortisone were administered parenterally at several dose levels for 10 days to groups of rats. The plasma cholesterol was determined as previously and the results are shown in figure 11. Thus, at relatively high dosages, cortisone elevated the plasma cholesterol level, whereas the physiologically occurring substances had little effect within this dose range.

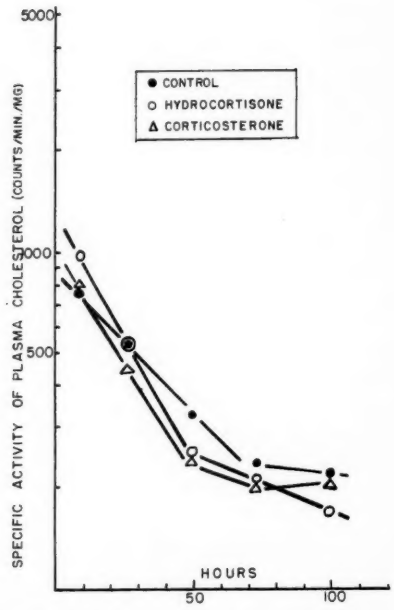

FIG. 12. Effect of adrenal cortical hormones on the biological half-life of plasma cholesterol in male rats.

Half-life of plasma cholesterol. The effect of corticosterone and hydrocortisone on the biological half-life of the plasma cholesterol has been studied in separate groups of young adult male rats. These substances (2 mg/100 g body wt.) were parenterally administered to 6 rats for 7 consecutive days. The biological half-life of the plasma cholesterol was then determined as detailed previously and the results are shown in figure 12.

Humans

The effect on the plasma cholesterol of the administration of cortisone to humans has been studied in many centres with somewhat conflicting results. The administration of this hormone in high dosage may be attended by a variety of severe side-effects; consequently the collection of plasma lipid data on normal subjects must be pursued with limitations and caution. For this reason, most of the information on the effects of cortisone on the plasma lipids has been obtained incidental to therapy on heterogeneous groups of patients receiving variable amounts of the substance for different periods of treatment. Adlersberg, Schaefer and Drachman (1) showed

that cortisone produced an elevation of the plasma cholesterol in severely ill patients. On the other hand, Conn, Vogel, Louis and Fajans (14), and Oliver and Boyd (34) found that cortisone lowered the plasma cholesterol in hypercholesterolaemic males. These apparent discrepancies have been resolved by the studies of Skanse, von Studnitz and Skoog (38) who concluded that cortisone caused an initial depression of the plasma cholesterol with a subsequent rise on continued administration.

DISCUSSION

The plasma cholesterol arises largely by hepatic synthesis and is in a dynamic equilibrium with cholesterol in certain other sites in the body. Thus, a change in the rate of cholesterol synthesis by liver might be reflected by a corresponding alteration in plasma cholesterol concentration, or the change in synthetic rate might be offset by similar changes in cholesterol degradation, resulting in an altered turnover of cholesterol without any change in total sterol concentration.

Consequently, an alteration to the plasma sterol concentration may be due to changes in rate of synthesis or degradation, or to changes in some of the many factors which dictate the partitioning of sterol between the body compartments.

The sequence of events involved in cholesterol biosynthesis has been considerably clarified within recent years (6, 7, 15) and the biosynthetic pathway has been shown to include substances common to other well-known metabolic routes. Changes in intracellular concentration of some of these intermediates may be involved in the basic metabolic control mechanisms in sterol synthesis.

The results obtained in this and other studies suggest that endocrinological influences appear to exert only a 'fine control' over cholesterol metabolism in the rat and in the human. The experiments contained in this paper were designed to investigate some of the effects of the secretions of the gonads, thyroid and adrenal cortex on cholesterol metabolism in the rat and in the human. The reactions of the rat and the human were qualitatively similar in all the endocrinological procedures which it was possible to study in both species.

The removal of the ovaries from females of both species is attended by increases in plasma cholesterol concentrations which are of a similar relative order of magnitude. It was possible to study the biological half-life of the plasma cholesterol in the rat and from this curve determine the apparent relative rate of plasma cholesterol synthesis. The results would suggest that oestrogens tend to increase synthesis, decrease the biological half-life, and hence accelerate the turnover of the plasma cholesterol. The removal of the testes from male rats resulted in little change in the plasma cholesterol, while reports in the literature suggest that this situation in the human produces a decrease in the plasma cholesterol level (20, 21). The isotopic studies in castrated male rats indicate that, despite the plasma cholesterol concentration being little affected, the rate of plasma cholesterol synthesis was increased while the biological half-life was decreased.

The administration of natural and synthetic oestrogens to the male rat results in changes in the plasma cholesterol concentration. The most potent oestrogens in this respect appear to be hexoestrol and stilboestrol. Both synthetic substances produce striking depressions in the plasma cholesterol concentration and cause a very marked diminution of the biological half-life of the plasma cholesterol. All oestrogens so far examined in the human male produce depression in the concentration of the plasma cholesterol if given at an oestrogenic dose.

Ablation of the thyroid in the human results in a marked increase in plasma cholesterol concentration, whereas in the rat the increase is significant but small. The studies reported here indicate that the athyreotic rat synthesises plasma cholesterol at a diminished rate and the plasma cholesterol has an increased biological half-life. On the other hand, suppression of thyroid function by thiouracil causes a marked increase in plasma cholesterol concentration, and isotope studies show an increased rate of synthesis in the presence of an extended biological half-life. Considerable care must be taken in the interpretation of any experiment on cholesterol metabolism in mammals in which thyroidal suppression is achieved by this type of antithyroid drug.

The production of the hyperthyroid state in the male rat is accompanied by irregular changes in the plasma cholesterol concentration. Frequently, the plasma cholesterol level is depressed, but hyperthyroid rats are occasionally encountered with elevated plasma cholesterol levels. However, by dietetic alterations it is possible to produce a mildly hypercholesterolaemic rat which responds to hyperthyroidism by regular marked depression in the plasma cholesterol level. The administration of thyroactive substances, whether naturally occurring or synthetic, results in a decrease in the biological half-life of the plasma cholesterol associated with irregular changes in the apparent rate of plasma cholesterol synthesis.

Adrenalectomy in the male rat is followed by a decrease in the plasma cholesterol concentration and a decrease in the relative rate of cholesterol synthesis. The biological half-life of the sterol was not significantly altered by this procedure.

The administration of small doses of cortisone to human males produces a depression in the plasma cholesterol concentration, but at continued high dosage results in reversal of this effect. In the male rat, the administration of corticosterone or hydrocortisone over a wide dose range failed to influence the plasma cholesterol concentration but influenced cholesterol metabolism in so far as these corticoids increased cholesterol synthesis. Cortisone, on the other hand, when administered to the male rat produced an elevation of the plasma cholesterol level. This effect may be related to the fact that cortisone is not a physiologically-occurring corticoid in this species,

or to the higher relative potency of cortisone in some biological assays.

These studies would suggest that, in the rat and in the human, the gonads, thyroid and adrenal cortex exert significant influences on cholesterol metabolism. Secretions of all these glands affect cholesterol turnover but the mechanism by which this is achieved is still uncertain. Many other factors, such as the glycogen content of the cells, the dietary content of vitamins, trace elements, other accessory food factors and lipids, including cholesterol itself, have all been shown to influence sterol metabolism. As yet, few definitive experiments have been performed which pinpoint the precise mode of action of any of these factors involved in the metabolism of cholesterol, but the role of the reticuloendothelial system in this connection deserves greater attention.

It is exceedingly difficult to hazard an opinion on the mechanism by which drastic endocrinological procedures elicit metabolic responses. The precise mechanism by which hormones influence these processes requires considerable further investigation to correlate observations at the cell level with those prevailing in the intact animal.

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Panel Summary—Lipids in Health and Disease

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IN SUMMARISING THIS DISCUSSION, I should like to divide the material into six parts. First, we have had a number of questions on the absorption of lipids from the intestine. It is clear that there is now general agreement that hydrolysis of glycerides in the intestinal lumen is only partial. The extent of hydrolysis is probably between 30–50% but there can be quite a wide variation since there are adequate mechanisms for resynthesis of triglycerides within the intestinal cells. Again, there has been a narrowing of any continuing area of disagreement with regard to particulate absorption; some of the latest ideas on micelle formation may be found to link up with our earlier studies on the intraluminar emulsifying system. It is obvious that the critical size of particle determining visibility does not necessarily correspond with the critical size determining absorption into intestinal cells. As pointed out by Professor Bergström, the observation that the area just within the intestinal cell is remarkably free from visible fat particles is still unexplained. It is possible that some of the points with regard to the solubility of cholesterol made by Dr. Bronte Stewart may be relevant to this structure of lipid micelles and emulsion particles. A full understanding of the mechanism of lipid absorption from the intestine is important to the solution of many of the problems that we have discussed here today.

Secondly, there are other aspects of intestinal function that must be considered. One of great importance is the question of excretion of lipids. This is a vital question in relation to cholesterol and bile salt metabolism as Professor Bergström has so clearly indicated. This may also play an important part in the mechanism of action of polyunsaturated fatty acids on blood cholesterol levels. The evidence available so far is not entirely satisfactory and there is room for further work in this area. The question of lipid excretion also comes into the picture in some patients with steatorrhea; again, the source of excessive fatty material in many of these patients is not clear.

Thirdly, in considering the intestinal phase, attention must be paid to the intestinal flora. Professor Bergström stressed the importance of this in his paper. Intestinal flora are also of importance as a factor causing modification of faecal fats in both normal and abnormal subjects. It can also be shown that fats themselves may modify the intestinal flora. It is important to obtain precise information about alleged floral changes. The use of antibacterial agents will only alter the flora if they are sensitive to the agents used and rapid adaptation is to be expected. Some antibacterial agents may have other effects on the intestine and on digestive enzymes—for example, neomycin is said to inhibit pancreatic lipase. If antibacterial agents are used, therefore, the effects observed are not necessarily attributable to changes in the intestinal flora alone.

The fourth group of questions was concerned with food composition and its relationship to lipid metabolism. The effect of polyunsaturated fatty acids in the diet on lipid metabolism will be considered in the next section. Dr. Lang suggested that these unsaturated fatty acids may have deleterious as well as beneficial effects. There is, also, no doubt that unsaturated fatty acids may undergo deteriorative changes and that oxidation products may be harmful. Some questions were also asked about the significance of monoethenoid fatty acids in the diet. Present evidence suggests that their influence is mainly through effects on the physical properties of the fats; high melting point fats tend to have a low digestibility but this can be improved by the presence of lower melting point fats. There are some interesting indications in recent work that mono-olein is particularly useful in micelle formation and in triglyceride resynthesis; the significance of such findings must await further study. The question of information on lipids in Food Tables was raised; there would appear to be a real need for more detailed information but practical difficulties arise from the wide variations found in different foods. Many variations in the lipids of particular foods are brought about intentionally by the food technologist with a view to providing a food product that is more acceptable in a broad sense. These modifications are harmless to the consumer but should, of course, be kept under review. The influence of other nonfatty food materials was also discussed. In particular Dr. Hartroft described an immediate effect of the inclusion of choline in the diet; we made similar observations on fat absorption some years ago. Carbohydrate intake is also known to influence the distribution and utilisation of lipids.

The fifth area for discussion was the relationship of dietary fats to blood cholesterol levels, atheroma and ischaemic heart disease. Dr. Bronte Stewart and Dr. Hartroft have taken a fairly conservative line on these questions. They have each been responsible for important scientific contributions in this field and it is their work and that of other investigators which provides the basis for any correlation that may exist. The demonstration

of a correlation does not, however, mean that there is any causal relationship. Anyone with a medical training should appreciate how dangerous it is to use observations made in sick people, post-mortem studies or information from any other selected group as a basis for generalisations applied to the community as a whole. The evidence, especially as it relates to atheroma and ischaemic heart disease has, of necessity, to be largely *post hoc* in nature. For this reason it is wise to take a conservative view on the existence of causal relationships. It is clear that those people best able to judge do not advocate any change in general dietary habits at the present time. The use of polyunsaturated fatty acids to lower the blood cholesterol in patients with severe atherosclerosis or ischaemic heart disease may be regarded as an acceptable therapeutic approach. Even if this treatment proves successful, however, it does not follow that deficiency of poly-unsaturated fatty acids was a causal factor in these patients or that the earlier introduction of the dietary regimen would have prevented or modified the development of the disease processes found. There is clearly room for much more research in this field.

Finally, a number of questions were directed at Dr. Boyd on various aspects of the endocrine control of lipid metabolism. It is apparent, as Dr. Boyd pointed out, that various hormones may influence lipid metabolism. In the main, however, they appear to be a mechanism for fine adjustment in the normal subject. In endocrine disorders, however, marked changes in lipid levels in the blood and tissues may occur.

To conclude, there is every indication that the progress that is at present being made in the study of the fundamental mechanisms of lipid absorption and metabolism may bring us a clearer understanding of some of these important applied problems, such as those concerned with atherogenesis and ischaemic heart disease, in the not too distant future.

PANEL IV

Nutrition in Maternal and Infant Feeding

Chairman: Icie Macy Hoobler, USA
Moderator: F. W. Clements, Australia

Nutrition in maternal and infant feeding

F. W. CLEMENTS

Institute of Child Health, University, Sydney, Australia

INTRODUCTORY NOTE

The conduct of this session could be organized in a number of ways, each of which would be profitable to some of those present. In order that the greatest benefit might be derived by the largest number it is probably desirable to select a number of broad issues, using the contributed papers of the members of the panel as working documents supplying the basic information. It would be unfortunate if we were to devote the few valuable hours allotted to us to the discussion of minutiae which can always be most advantageously considered over a cup of coffee. If this principle is accepted the important task is the selection of the most suitable broad issues. Should time and opportunity permit, undoubtedly the most satisfying procedure would be an open discussion with those who plan to attend this session. Since these are, at this moment, unknown and since to wait until we gather at Washington would defeat the object of this paper, someone has to make a decision and this, presumably, is one of the tasks of a moderator. Early announcement of the plan of operation for this session should give intending participants the opportunity to think about the issues selected and gather together data from their own experiences to enrich the meeting.

Ideally, the issues selected should be of universal interest, should contain many unsolved problems and should promote such discussion that a few signposts can be erected indicating possible avenues of fruitful work in the future.

EFFECTS OF DIFFERENT LEVELS OF MATERNAL NUTRITION

INADEQUATE NUTRITION of infants reveals itself in a number of forms: *a*) as undernutrition, exemplified by failure to thrive; *b*) as specific deficiency states produced by the dominating deficiency of one or more specific nutrients, for example, protein (kwashiorkor); thiamine (infantile beri beri), ascorbic acid (scurvy); vitamin A (xerophthalmia and keratomalacia); vitamin D (rickets).

Where are the beginnings of the departures from normal which end as the various deficiency states? There is some evidence that, for some of these, the process begins in utero, for example, scurvey (7, 8, 11, 20) and rickets (13, 14) do appear, albeit rarely, in the newly born. However for the great mass of infants who develop deficiency diseases the first diagnostically acceptable clinical signs do not appear until some time after birth; nevertheless it is possible to develop a convincing argument that for many of these infants the beginnings could occur in foetal life. The material for this argument comes from the few studies of nutrition of the mother during pregnancy.

In the healthy well-nourished woman pregnancy is associated with some weight gain, although there is some disagreement among obstetricians as to what is the most desirable. Leitch (10), reviewing the weight gains of women in Aberdeen, concluded that for the hypothetical average Scottish woman in good health and nutrition a gain in weight of 12.5 kg is to be expected. This gain can be partitioned as follows:

	Kg	
Total weight gain	12.5	
Product of conception	5.0	
Foetus		3.23
Placenta		0.64
Liquor Amnii		1.44
Maternal net gain	7.5	
Components of maternal gain		
Water	6.0	
Plasma		1.2*
Extravascular extracellular		2.2*
Intracellular		2.6
Protein	1.5	

* Quickly lost after parturition.

In the early stages of the post-partum something like 4.0 kg remains (19) and is ultimately lost by the great majority of women over the ensuing 6 to 12 months providing their diet is good. (See paper by Gopalan in this series for reference to effects of poor nutrition in the post-partum on body weight.)

A significant percentage of women, who have easy access to a plentiful supply of food (commonly in all socio-economic groups in prosperous countries, frequently in members of the higher socio-economic groups in less prosperous countries), gain more weight than this average amount. In most instances this is due to the consumption of too many calories and an imbalanced diet with high intake of carbohydrates and fats. It is now generally accepted that an excess gain or normal weight gains on a markedly imbalanced diet are accompanied by increased incidence of pre-eclampsia and the toxaemias of pregnancy.

It is important to know whether diets which produced these abnormalities of pregnancy are associated in foetal and infantile malnutrition.

In the less prosperous countries and among certain sections of the population in prosperous countries many

TABLE 1. *Percentages of Women Making Specified Weight Gains in Pregnancy*

	Formosa %	Ceylon %
8–11 kg	25	10
5–8 kg	25	32
3–5 kg	30	30
Less than 5 kg	15	23
Lost weight	5	5
No. of records	130	88

TABLE 2. *Variations in Level of Storage of Some Metabolites in the Full-Term Infant*

	Minimum	Maximum	Mean	Reference
Calcium content of full-term newborn infant	13.1 g 5.0 g/kg	27.9 g 10.3 g/kg	23.7 g 8.1 g/kg	5
Vitamin A content of liver of full-term newborn infant	25,280 IU	60,755 IU	39,994 IU	6
Nicotinic acid content of liver of newborn infant	780 g %	3,560 g %		16

women gain much less than 12.5 kg during pregnancy. For women of small stature a smaller gain is probably acceptable. This point could, however, be debated.

In 1957 the author collected information about the weight gains of women attending a number of health centres in Formosa and Ceylon. The percentages who made specified weight gains during pregnancy are shown in table 1. It will be seen that a significant number of women failed to gain an amount of weight equal to the weight of the baby they produced and that a few women actually lost weight.

What is the effect of small weight gains by pregnant women upon their own metabolism and that of the foetus?

What is the effect of maternal undernutrition on lactation? (See Gopalan's paper.)

There is some evidence that the foetus is able to store nutrients over and above those needed for structural formation and growth. A number of investigators have shown that the amounts of some nutrients present in apparently full term healthy foetus vary widely, even up to 100%, as the figures in table 2 show.

Is there a priority for the distribution of nutrients consumed by the pregnant women?

As a point of departure for discussion, it is suggested that if there is an order of priority it might be as follows:

Order of priority	Destination of nutrients
1	Foetal growth
2	Uterus and placenta
3	Blood volume and haemoglobin (as iron and protein are available)
4	Breast enlargement
5	Foetal storage
6	Maternal storage

Perhaps it might be argued that numbers 5 and 6 should be reversed.

Would variation in the level of nutrient storage during foetal life explain the occurrence of deficiency disease in only a fraction of the infants in any locality where the conditions of life, including food intake and child rearing practices, appear on the surface to be uniform?

For example, does variation in the level of vita Amin and carotene storage during foetal life depend upon the level of intake of these two nutrients by the mother during pregnancy and does the level of storage determine the age of onset of the various ocular manifestations of vitamin A deficiency and so the tissue involved and the form the signs take?

Is there any information about protein storage in foetal life and its relationship to the occurrence and age of onset of kwashiorkor?

If there are variations in foetal storage, does it mean that the prevention of frank deficiency diseases in infants and young children can be prevented by a significant improvement in nutrition during pregnancy?

SIGNIFICANCE OF SOCIAL AND EMOTIONAL FACTORS

The standards of child rearing and the attitudes towards infant care and management vary widely from one community to another, in the same community over a period of time and in different classes within the same community. These variations are frequently reflected in the infantile mortality and morbidity rates. Peller (17) has shown that during the 16th, 17th and 18th centuries the post neonatal infantile mortality in the ruling families of Europe was less than one-third of that prevailing in the general population. Peller (18) points out that in the 17th century the ruling families "knew how to prevent summer diarrhoea and the summer peak of infant mortality, while the physicians attending the general population were still wrestling with this problem at the turn of our century."

We accept the fact that differences exist in attitudes to child rearing but how do they arise, what in terms of day to day functioning are they and how might variations affect the life of the infant and child? These differences can arise from: i) The community's attitude to children, including their place in society, the amount of time women are permitted to devote to child management, the recognition of the effects of pregnancy and lactation on the women with their appropriate release from the labour force; (see paper by Jelliffe and Bennett). ii) The attitude and feelings of the individual mother; this may be an expression of the community attitude or it may arise within the woman herself as an expression of her own personality.

Attitude of community. The community's attitude is seen clearly in the attitude of the ruling families of Europe in the 16th to 18th centuries—the survival of the largest number of children born alive was important for the maintenance of the family and the expansions of its ties through marriage leading to enhanced power.

Two island groups of primitive peoples offer interesting material for comparison of the practices in infant rearing arising out of the attitudes of the two communities toward children and the consequences. Maloney (12) has described the mother-child relationship amongst the people of Okinawa, and Du Bois those amongst the Alorese (2).

The Okinawa is well mothered. This starts directly after birth when he is offered "well nippled breasts that are copiously filled with milk". Later he is offered the breast whenever he is hungry or disturbed. This allays his anxiety and affords a sense of protection and security. It is an interesting aside that this is a common practice among many primitive peoples. I have repeatedly noticed that an infant who is disturbed by the arrival of strangers can be quietened immediately by the mother forcing the breast into its mouth. Maloney believes that the Okinawa mother does everything in her power to prevent early frustrations. The infant is carried around with the mother in a shawl for upwards of two years, during which time it receives constant comfort and attention. The outcome is an unspoiled child capable of self discipline and harmonious social cooperation. Maloney was greatly impressed with the manner in which the Okinawas, as a group, withstood the severe bombing and shelling with its destruction of home crops, etc. The physical and emotional breakdown rate was extremely low. This he attributed to the care bestowed on the child in its early years.

The young Alorese is not so well mothered. During the first four to six days after birth the mother devotes herself exclusively to the fondling and feeding of the infant. From the sixth day onward the infant is given, in addition to human milk, premasticated foods including roasted bananas and vegetable gruels. When the mother returns to work on about the 14th day after the birth, the infant is left during the day in the care of an older child or relative. It is not customary for the infant to be taken on the back of the mother. There is great variation in the amounts of attention the child received. Although attempts are made to satisfy hunger, these are not satisfactory, and the child does not have the opportunity to form strong attachments to the mother. Kardiner (9) in his analysis of the Alorese culture summarised the situation by suggesting "the child may be nourished but it is not nurtured." During the period of helplessness the maternal care fails. Similarly the pattern of care throughout childhood and adolescence tends to develop frustrations. The basic personality of the Alorese is characterised by anxiety, suspicion and mistrust.

In both cases the patterns described represent the common practices in the localities: there are, of course, individual variations which result from differences in the degree of emotional maturity of the mother, her sense of security, social acceptability, etc. These factors operate in every community.

Mother's personality. The mother interprets the community's attitude according to her own personality; this will tend to either reinforce or extinguish the extremes. To this she may also add variations which arise from her own personality, which has been determined largely by her own life experiences, of which undoubtedly the most important is her relationship to her own mother. If this has been satisfying she will have laid the foundations for a capacity to make relationships with people. This will enable her to make pertinent observations of her infant's needs and to take steps to meet these, and also to have empathy towards her children. A mother can establish good relationships, or reject her child.

We must also accept that she may have to neglect him for material reasons (e.g. food gathering), which perhaps is not as devastating as psychological rejection.

Variations in child rearing practices. The young infant has few basic needs, of which satisfaction of hunger is probably the most important.

Anna Freud (4) has drawn a dramatic picture of the tense, crying, hungry child who is expressing frustration and rage because he cannot tolerate the pain (or feeling) in his stomach and has contrasted this with the contented recently fed infant.

The baby who is seldom required to experience the acute pang of hunger and is able to obtain, without undue effort, the required amount of food at each meal can easily develop pleasant and satisfying feeling both toward food and the mother, thus laying the foundations for good and satisfying relationships with his mother. Later these spread out, as Erikson (3) has stated, to feelings of trust towards the community at large.

If the mother is tense, preoccupied, secretly rejects the baby, he might get enough food but the feeding experience is unsatisfactory; it might be for him decidedly unpleasant. He soon comes to associate food and feeding with a feeling of inadequate gratification of his total bodily and emotional needs. These total body feelings are reflected in his subsequent attitude to food and feeding, around which he may develop an attitude of indifference and even aversion, and, following through with Erikson's notion, he develops an attitude of distrust.

The dominance of the stomach in early infancy as a sensory organ makes infant feeding the dominant variable in child rearing practices which affect nutrition.

There are, however, other factors. Bowlby (1) has recently advanced a new hypothesis to explain the infant's tie to his mother. He suggests that there are five instinctual responses which together build this tie; these are sucking, clinging, following, smiling and crying. The infant must be given the opportunity to satisfy fully the first three and must elicit appropriate responses from his mother to his early attempts to smile and to his crying if his emotional development is to proceed smoothly to fruition.

Each feeding occasion should be, in its entirety, pleasant and satisfying. Not only must the supply of food be adequate, easily obtained and fully digestible, but the mother must have the time and opportunity to give the baby satisfying human contact which gratifies his need for clinging and support.

According to Bowlby, sucking, both nutritive and non-nutritive (of which it is suggested the infant needs at least two hours a day) is closely related to the other four instinctual responses, of which in early infancy crying is of great importance. The mother (or satisfactory mother substitute) must be there for the infant to cling to and the experience must be gratifying.

Later he will have a strong desire to follow his mother, first, before he is mobile, with his eyes and later, as a toddler, on foot. The mother must be there for this to happen, and he must get satisfaction from the visual and tactile contact. Because there is probably a close linkage between these five instinctual responses, failure to find satisfaction for one of them can affect others, and thus the infant who is frustrated in his attempts at following may show this in his reactions to food and feeding.

Possible effects of social and emotional factors on nutrition of the pregnant woman and the infant. Wolf and Wolff (21) have shown that there is a close relationship between the emotions and the functioning of the gastro-intestinal tract. Strong emotions, particularly fear, hate, anxiety, bring about changes in the intestinal mucosa which must interfere with the digestive processes.

Is it likely that poor development of one or more of the instinctual responses in some infants is associated with poor functioning of the gastrointestinal tract, with the subsequent interference with digestion of foodstuffs and the absorption of nutrients?

Is it possible that some types of food, e.g. animal and fish muscle and vegetables are more likely to be affected than others—say human milk? (See papers by György & Platt in this series.)

EFFECTS OF SPECIFIC DIETARY PATTERNS UPON BOTH PREGNANT WOMEN AND INFANTS

Pregnancy. One authority (15) has laid down the Recommended Dietary Allowances for the second half of pregnancy as follows:

Calories	2500–2600	Thiamine	1.3 mg
Protein	78 gm	Riboflavin	2.0 mg
Calcium	1.5 gm	Niacin	20 mg
Iron	15 mg	Ascorbic Acid	100 mg
Vitamin A	6000 IU	Vitamin D	400 IU

It is clear from Gopalan's paper that a significant number of women during lactation do not receive a diet which will provide anything like these amounts of calories and the various nutrients, so it is reasonable to expect that diet during pregnancy was also deficient.

It is not the purpose here to discuss deficiency of nutrients or even of calories but to raise the question of the relative proportions of fat, carbohydrate and protein which contribute to the calories. Protein nutrition has received a great deal of attention in recent years and it is not proposed to extend further this discussion in this paper, although participants may have pertinent information to contribute.

Is the ratio of fat to carbohydrate in the diet of the pregnant woman important? If so, what is the minimal intake of fat consistent with good nutrition and good health?

Infancy. The amount of fat in the diet of infants may be of much greater importance. In breast milk, approximately 61% of the calories are supplied by fat. In undiluted cow's milk, approximately 50% of the calories are supplied by fat. In many of the formulae based on diluted cow's milk, the calories supplied by fat often do not exceed 30% of the total.

Almost all the weaning diets, other than animal milk, traditionally used throughout the world, are based upon animal broths, cereals, root crop and vegetable purees, the fat content of which is very low. We know that the great majority of healthy infants can tolerate and digest mixtures containing quite a high percentage of fat, and it would seem from numerous published works that infants suffering from kwashiorkor respond well to diets containing appreciable amounts of fat.

What is the significance of a regular diet low in fat in infancy upon nutritional status generally and the onset of deficiency diseases in particular?

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Orientation in infant feeding

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SEEN FROM A GLOBAL ANGLE, without the complicating influence of advanced civilization and its achievements in public health and in rational nutritional education, it is safe to state without fear of contradiction that human milk is for the human infant and cow's milk is for the calf! Any other food replacing human milk is a substitute in the diet of the human infant.

In many technically underdeveloped countries with poor general hygiene, widespread intestinal infectious diseases, with interfering tabus and superstitions, breast-feeding is the best and, in many parts of the world, the only insurance for the survival of young infants. This observation and conclusion should not imply, however, that the beneficial effect of breast-feeding consists only in the prevention of contamination, in break of normal aseptic technique which is more likely to occur in bottle-fed infants.

HUMAN MILK

Animals differ in their growth-rate and, in consequence, necessarily in their requirements for calories and body-building food constituents, in the first place—protein, from human infants. Furthermore, rat, the most commonly-used of animals in experimental nutritional research, happens to be sensitive to the high content of lactose in human milk. Thus, it is understandable that human milk received in the past no particular attention by experimental nutritionists working with animals. Since the milk of primates is very similar (1) in its composition, at least with regard to the major constituents (protein, lactose, fat, salts) to that of human milk, primates might offer a good opportunity for long-term nutritional studies with human milk. Fractions of human milk may be profitably used even for smaller laboratory animals, as has been recently successfully demonstrated with the proteins of human milk on rats (2).

On the other hand, there exists sufficient clinical empirical evidence that human milk, as such, has distinctive properties which may reflect themselves in the infant fed human milk. Specifically, infants fed human milk distinguish themselves—at least in two respects—from those fed cow's milk. First, breast-feeding reduces both morbidity and mortality rates, especially the latter. It is of special interest that infants fed human milk, as compared with those fed cow's milk, demonstrate higher resistance not only to intestinal disorders but also to respiratory diseases, including such complications as otitis media. Those of us, like myself, whose pediatric practice dates back many decades, will have no hesitation to testify in favor of breast-feeding in the prevention of infectious diseases and as, at that time, the best therapeutic diet for infants with chronic and often very severe acute pyogenic infections. Similar observations were made in chronic intestinal disorders or severe malnutrition combined with chronic infection. In general, this effect of human milk in a variety of infections was ascribed not so much to the presence of specific antibodies but to the action of unspecific factors of unknown origin. Transfer of immune bodies from ingested human milk through the intestine into the blood is a negligible factor in resistance to disease if it occurs at all.

Improved general hygiene and the use of antimicrobial agents have, of late, obscured the superiority of human milk over cow's milk in regard to the figures of morbidity and mortality. Recent statistical studies, however, published from by no means underdeveloped countries like the United Kingdom and Sweden, indicate that these differences may be demonstrated, not only under poor, but also under good hygienic conditions (3–5).

The unspecific factors surmised in human milk as related to resistance to disease have not yet received intensive experimental study. They might act directly on organs such as the reticulo-endothelial system or the so-called pituitary-adrenal axis in analogy to bacterial extracts or to purified lipopolysaccharides obtained from bacteria (6–7). In this instance, we would deal with an unspecific immune-reaction. Or, constituents of human milk might enhance the production of specific antibodies. One could imagine that in a chronic staphylococcal infection, the antigen liberated from dead staphylocci would in amount not suffice to stimulate antibody production. This might become possible through a synergistic action of a constituent of human milk (lipid?) or, indirectly, through symbiotic action of metabolites produced by the intestinal flora which, in itself, is specific and characteristic for the normal breast-fed infant.

This brings us to the second distinctive feature regarding infants fed human milk as compared to those fed cow's milk. In contrast to the acid reaction of the feces of normal breast-fed infants, the pH of the feces of infants given the usual cow's milk formulae falls in the

neutral or alkaline range. Unlike the mixed intestinal flora of infants on cow's milk formulae the intestinal flora of healthy breast-fed infants is characterized by the prevalence of a particular species of *Lactobacillus*, namely *Lactobacillus bifidus* (8, 9). A gram-stained fecal smear obtained from healthy breast-fed infants appears to be almost uniform, as if it would represent a pure culture of gram-positive rods characteristic of *L. bifidus*. On the average, the proportion of gram-positive rods is around 98%, with only slight variation, in normal breast-fed infants.

In the past and even recently, it has been widely claimed that the acid fecal reaction, perhaps together with the prevalence of *L. bifidus* flora, may be beneficial to the human infant, through suppression of pathogenic or otherwise harmful intestinal bacteria, such as coliform and other proteolytic, putrefying microorganisms, or by improved symbiosis between the intestinal flora and the host organism or by some other obscure effects. In addition to the low fecal pH, the specific fermentation products of *L. bifidus*, i.e., lactate and, even more, acetate and formate furnish a specific and distinct contribution to this antibacterial effect (10). From this point of view, the effect of acid fermentation is comparable to that of antibiotics. In the language of the past century, an aciduric *L. bifidus* flora in the intestine should counteract constipation, putrefaction with its supposed sequela of 'intestinal autointoxication'. In our own extensive studies of a large selected group of *L. bifidus* strains, we have found no indication for the production of 'specific' antibiotics by *L. bifidus*, which could have acted either locally or, after their absorption, systematically as antimicrobial agents, and might have linked the specific *L. bifidus* flora in the intestine with the high natural resistance of the breast-fed infant.

The establishment of a particular intestinal flora is not due primarily to the presence of the microorganism in question, but more to the existence of favorable cultural conditions or, in other words, to the medium as it is found in the intestinal lumen. The milieu depends chiefly on two factors: the endogenous contribution of the intestine as such, and the exogenous factor of the food ingested. With regard to the first, the endogenous factor, it is of special interest that the bifidus flora of the breast-fed infant may be considerably suppressed as the initial sign of disease of varied etiology without any concomitant, quantitative or qualitative change in the exogenous factor of food ingested. The second, the exogenous factor, leads us to the study of the chemical composition of human milk as compared with cow's milk.

In the course of investigations on the metabolic requirement of various strains of *L. bifidus* isolated from the feces of young infants, we encountered in our laboratory an apparently specific variant of *L. bifidus* (11). On our regular medium, this newly-isolated strain has shown at primary isolation only scant or no growth. Further propagation of this new variant of *L. bifidus* which was named *L. bifidus* var. pennsylvanicus (12) was achieved

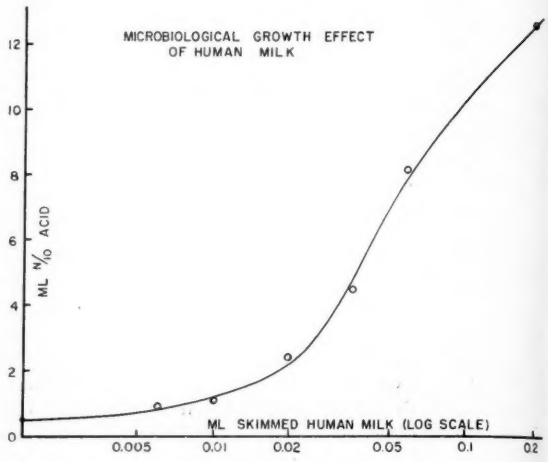

FIG. 1

in consecutive subcultures when human milk was added to the medium (11). With increased supplements of human milk to the original liquid medium, acid production as an index of bacterial growth increased expotentially towards a maximum (fig. 1), indicating that a constituent of human milk must be an essential growth factor for *L. bifidus* var. pennsylvanicus (11).

With average samples of human milk, half-maximal growth was reached with about 0.06 ml of whole or skimmed milk added to 10 ml of the basal liquid medium. In contrast, cow's milk was practically inactive. In the example shown in table 1, the ratio of activity of cow's milk to human milk was about 1:50. A similar ratio of activity, with an average of 1:40, was found with other samples of human milk and cow's milk. Thus, with regard to this microbiological growth-factor, the activity of cow's milk is definitely of a lower order of magnitude than that of human milk.

The 'bifidus factor' in human milk is not destroyed or altered in its activity by autoclaving. Human milk ash was inactive.

In further studies, the activity of human milk was compared with that of the milk of other species (table 2). The average relative activity was the highest for human colostrum, closely followed by rat colostrum, then by human milk, rat milk, cow's milk and sow's colostrum. All the other milks tested, in particular the milk of ruminants, such as cows, sheep and goats, have shown only very slight activity (13). The highest titre of the bifidus factor in early milk, not only in cow's colostrum but in human colostrum, rat colostrum and sow's colostrum may have a special significance. Chemically, the bifidus factor belongs to the group of N-containing carbohydrates. In human milk, the presence of great variety of such neutral or acidic oligo- and polysaccharides has been demonstrated (14, 15). Their total quantity in fresh human milk may be estimated to be around 0.7%, which is by no means negligible an amount with respect to total solids.

TABLE 1. *Assay of Human and Cow's Milk*

Supplement (per 10 ml of medium)		Titration ML. n/10 NaOH
None		0.4
Human milk	0.02 ml	2.2
(skimmed)	0.06	7.9
	0.2	12.5
Cow's milk	0.1 ml	0.6
(skimmed)	0.3	0.8
	1.0	2.3

TABLE 2. *Activity of Human Milk as Compared with Milk from Other Species*

Species	Activity	One Unit in	Relative Activity
Guinea pig	0	—	—
Cow	(+)	2.5 ml	2.5 ml
Sheep	(+)	2.5	2.5
Goat	(+)	2.5	2.5
Mare	+	0.5	12
Sow	+	0.4	15
Cow colostrum	++	0.15	40
Rat	++	0.13	45
Rat colostrum	++	0.03	200
Human	++	0.06	100
		(0.02 to 0.15)	
Human colostrum	++	0.02	300
		(0.01 to 0.03)	

The nutritional importance of these N-containing oligo- and polysaccharides is still shrouded in mystery. Chemically, they give human milk a special position, particularly in comparison to cow's milk.

For some strains of *L. bifidus* pantethine, thymidine and essential fatty acids represent essential or secondary growth stimulants (16–18). A strepogenin-like growth-promoting factor (16, 19), called also bifidus factor 2 (20) has also been claimed for some special strains of *L. bifidus*. For *L. bifidus* var. pennsylvanicus menadione and other vitamin K analogues have been recognized as supplementary growth-factors (21).

An inhibitory effect of *L. bifidus* var. pennsylvanicus on regular strains of *L. bifidus*, and, to some extent also, on *Escherichia coli* has been demonstrated (22). Such observations made through *in vitro* tests suggest how a well-established *L. bifidus* var. pennsylvanicus flora might suppress the growth of less desirable organisms and thus contribute to the normal ecology of the intestinal flora.

All these studies have shed not only light on hitherto unrecognized specific chemical qualities of human milk, but also on the possible relation to the normal intestinal flora of the breast-fed infant.

Petuely (23) claimed that lactulose, the keto-analogue of lactose, added as carbohydrate to dilute cow's milk may promote the establishment of a characteristic bifidus flora in infants. Since lactulose is not present in human milk, it cannot be the physiological bifidus factor.

Scepticism against the overestimation of the differences in the intestinal flora between breast-fed and artificially fed infants is best countered—or at least shaken—by reference to the very interesting, not well-known disease-entity, familiarly named acrodermatitis enteropathica. The disease is characterized by vesicular dermatitis around the body orifices and the distal parts of the extremities, with multiple paronychia on hands and feet, diarrheal attacks and other digestive dysfunction.

The disease usually appears after weaning and without treatment—is usually fatal. Recently, we observed the condition in a very young infant, only a few weeks old, fed cow's milk formula since birth. Brandt, in 1936, stated (24) that "among all therapeutic experiments that have been instituted, the treatment with mother's milk is the only one that has any demonstrable effect. After addition of mother's milk to the diet, there has been an increase of the baby's weight from a previously almost arrest of growth, and in particular—the general condition has shown a distinct improvement. . . .Diet free from cow's milk or addition of goat's milk were ineffective."

In our own observations, human milk has also shown beneficial effect but, unfortunately, the supply was exhausted before complete remission was obtained.

More recently, the intestinal antiseptic Diodoquin has proved to be a very effective remedy in the treatment of acrodermatitis enteropathica (25).

The nature of this intriguing condition is unknown; it is probably based on an inborn metabolic error with its original site in the intestinal flora and, in further consequence, in the inability of the organism to detoxify bacterial products absorbed from the lumen of the intestine.

All these effects, although not completely elucidated, point toward specific chemical properties of human milk. Nevertheless, one finds many adherents of breast feeding who tend to disregard the direct nutritional value of human milk, basing their recommendations for breast-feeding chiefly on psychological considerations, i.e., mother-infant relationship. The importance of emotional factors in breast-feeding should certainly not be minimized, but at the same time also not exaggerated to the detriment of purely nutritional factors inherent in human milk.

In many countries, especially in the technically highly-developed, breast-feeding is rapidly declining. The apparent success of artificial feeding, certain cultural-anthropological factors, the availability and intense promotion of proprietary infant foods contributed to this discouraging development. According to a recent statistical study (26), never more than 25% and as low as 12% of the newborn infants leaving the Hospital Nursery were exclusively breast-fed, in various parts of the United States in 1956.

The composition of human milk is not absolutely constant with regard to the content of protein, fat and even the exact chemical nature of fat. However, under not excessive conditions, these variations, although significant, exhibit, with the exception of early milk (colostrum), a regular distribution curve more or less independent from the diet of the mother. The crude composition of human milk is well exemplified in the average by the figures given in table 3. Various samples

TABLE. 3 *Composition of Human Milk and Cow's Milk*

	Human Milk %	Cow's Milk %
Protein (N × 6.25)	1.2	3.5
Lactose	6.6	4.0
Fat	3.5	3.5
Ash	0.2	0.7
Calories	65	65

of human milk obtained from undernourished lactating mothers in India and Jamaica have furnished in our laboratory quite similar analytical data. Undernutrition of the lactating mother will usually reduce the total amount of milk produced. This will lead to marasmus of the breast-fed infant. It is questionable whether on exclusive breast-feeding kwashiorkor could develop.

COW'S MILK VS. HUMAN MILK

Even in the earliest developmental phase of scientific pediatrics, 50–60 years ago, there were teleologic considerations which furnished the foundation and leading principle for artificial feeding of infants. In comparing the crude chemical composition of human milk with that of cow's milk (table 3), it is not surprising that the higher content of cow's milk in protein and salts, which are the specific 'body-building' constituents, compared with the corresponding average analytical data for human milk, has been related by the pioneers of modern pediatrics at its inception to the more rapid growth and greater apposition of tissue in the calf than in the human infant. The additional assumption, not supported by scientific facts, but only by clinical observations and 'impressions' have been made that too much protein and salt as present in undiluted cow's milk may be harmful to the young infant. Czerny's 'Milchnährschaden' (milk dystrophy) was looked upon as the result of overfeeding with milk, mainly protein and salts. Today, it has been customary, perhaps with unwarranted superficiality, to see in this syndrome the result of a qualitatively incorrect dietary regime, such as that of lack of iron, vitamins, supplementary feeding (vegetables, meat, etc.). Of course, this possibility, being the result of consecutive nutritional research, could not have been even considered at the beginning of the century, and dilution of cow's milk as initial formula for the young infant was strongly favored and widely accepted. One part of cow's milk diluted with two parts of water represented the first step in adaptation of cow's milk to human milk, at least with regard to protein and salts. The reduced caloric density of this formula was compensated in part with the addition of carbohydrate. But still it became often difficult to cover even alone the caloric requirement of the infant with such dilute formula, necessitating much higher intake in volume than the infant could cope with. This initial step in artificial feeding with a mixture of milk-water-sugar has undergone gradual changes during the consecutive decades up to the present time. The danger of undernutrition with the too dilute one-third milk has led to the use of the more concentrated ½–⅔ milk-water-sugar mixture, as initial formula, with satisfactory caloric density.

With the progressively increasing intrusion of cow's milk formulae in pediatric practice, some difficulties have arisen such as diarrhea, vomiting, 'colic', unsatisfactory gain in weight, etc. Acidulated milk, introduced in Holland, then used in Germany in form of buttermilk, has been considered by prominent pediatricians as the answer to better artificial feeding. Such acidulated formulae were in wide acclaim in the United States 10–20 years ago, but at present are used but sparingly.[1] In tropical and warm countries, and in regions with less well-developed hygienic conditions and with a dearth of refrigeration facilities, acidulated milk still may be advisable. The high acidity acts as a preservative and may counteract, certainly to some extent, secondary bacterial contamination. In reverse, it may be stated that the greatest benefit in successful artificial infant feeding has been achieved by following proper aseptic rules: pasteurization, autoclaving and other related procedures. One is even justified to state that with good aseptic care, it is difficult to cause clinically demonstrable harm to a normal infant kept on any feeding regimen, providing the regimen covers the minimum requirements for all essential nutrients.

The trend in modern pediatric practice is toward maximal growth. In this connection, one could legitimately ask: Can physicians be sure that accelerated weight-gain and overfeeding are only temporary interludes and will have no lasting effect which might manifest itself, perhaps years or even decades later, in adult life? Be that as it may, there is no reason to believe that maximal weight gain and accelerated maturation in infancy should be the optimal nutritional goal.

Based on short-term clinical observations, artificial infant feeding as it is used today under strict rules of hygiene yields good results: Cow's milk formulae, mixed feeding, meat, cereals etc., even when, as is now customary, at an early age, vitamin supplements will support satisfactory growth and development in a normal infant.

A quotation from the recent report of the Committee on Nutrition of the American Academy of Pediatrics summarizes the present status very well: "The practice produces neither beneficial nor harmful results but rather attests the adaptability of the baby to the whim of his caretakers."

All these developments and tendencies, supported by the increased use and distribution of canned evaporated milk and of proprietary concentrated milk preparations, determined the wide popularity of concentrated milk formulae. The large selection of other commercially available baby food (cereals, vegetables, meat products, etc.), opened the way to the early use of supplementary food in the infant dietary. The great majority of artificially-fed infants in the United States is kept on such feeding schedule which implies a high intake of protein,

[1] In premature infants, lactic acid milk produces acidosis and may interfere with normal development (27, 28).

salts and in many instances also of calories. Even very young infants are receiving, nowadays, milk-water-sugar mixtures which will furnish a daily intake equivalent to 100 calories, 3.5 grams (or more) of protein per kg body-weight. This figure (3.5 grams) was the previously (1953) recommended daily allowance of the Food and Nutrition Board of the National Research Council, for infants. Premature infants are receiving even higher amounts of protein, 4.0–6.0 g or more daily.

If one would adhere to the term 'allowances' as the Report of the Food and Nutrition Board interprets it, one could perhaps acquiesce in the acceptance of the feeding scheme for infants as generally used today. . . "Allowances are designed to maintain good nutrition in healthy persons in the United States under current conditions of living and to cover nearly all variations of requirements for nutrients in the population at large" (29) This conditional acceptance would, however, imply that the allowances as stated are based on scientific facts and not prompted in the first place by convenience or by recognition of existing customs and practices. It appears, however, that *a*) Convincing scientific support for the recommended daily allowance of 3.5 grams of protein in infancy is not available. *b*) 'Allowances' are often mistaken for 'requirements.' There is a frightening widespread shortage of protein over a large part of the inhabited globe, especially in the tropical belt. Protein malnutrition is rampant in these areas, particularly affecting infants in the post-weaning period. From a general, world-wide, socio-economic point of view it is imperative to remain staunch and exact with our dietary standards and to avoid exaggerated claims. One should guard even from the semblance of 'double moral,' allowing "for the maintenance of good nutrition in the United States" (20) high protein intake, when such goal is outside of reach for the majority of the world's population and may not even be necessary, perhaps even desirable. *c*) The most telling argument against the figure of 3.5 g of protein per kg and day for infants was the fact that with such allowance, breast milk will have been considered 'sub standard', since infants fed only on breast milk could practically never have a daily intake of 3.5 grams of protein. Or else, they must be grossly overfed with a caloric intake of over 200 calories per kg and day (instead of the normal 100–120 calories). Obviously this dilemma could have been solved by giving separate allowance-figures for breast-fed and artificially fed infants. (2.0 versus 3.5 grams of protein.) Such distinction between human milk and cow's milk would imply that *a*) the protein of cow's milk is inferior in biological value to that of human milk, or *b*) cow's milk, at least its protein, may not be utilized and assimilated in a manner comparable to the protein of human milk. Recent experimental studies in rats revealed, however, only a slight superiority of human milk over cow's milk (30), or statistically insignificant difference in the nutritional indices of protein efficiency ratio, protein retention efficiency, net protein utilization, biological value and true digestibility between protein of human milk and cow's milk (2).

Also, in infants, no significant difference was found in the utilization of protein offered in human milk or cow's milk. Even in premature infants kept on low protein rations (about 2.5 grams of protein in form of cow's milk per kg and day), the results were perfectly satisfactory and statistically not different from those observed on a high protein diet (6.0–8.0 grams per kg and day, again in form of cow's milk), at least not with regard to weight gain, total serum protein, serum protein fractions, Hb and erythrocytes. The reduced concentrating power of the kidneys in these young premature infants manifested itself in higher figures for serum-phosphate and BUN compared with those found in infants on a low protein formula. The high total phenol excretion in the urine of infants fed the high protein formula indicated increased catabolism of aromatic amino acids (31). In severe protein malnutrition, the protein of human milk is apparently better utilized than that of cow's milk (32).

For practical purposes, one may state that the protein of human and cow's milk do not differ appreciably—at least for normal infants—in their biological value. Thus, it would be unjustified to set even the figures of 'allowance' for protein higher for the young infant than it could conceivably be covered by human milk. This explains the fact that in the last edition (1958) of Recommended Dietary Allowances (29) the previous figures for protein-allowance (3.5 grams per kg and day) has been omitted and no 'allowance' was indicated for the first year of life. It is not surprising that in the light of this development and with improved technology, adaptation of cow's milk to human milk has made considerable progress during the last few years. This is the natural sequela of modern technological progress. The early use of milk-water-sugar formulae was necessitated by the difficulty of dispersing, in proper homogenization, fat in such milk dilutions for home consumption. During the last few decades, canned milk and other canned baby foods became commercially available and are—at least in technically highly developed countries—preferred to fresh milk or to baby food prepared fresh in the kitchen. Enrichment of dilute milk-water-sugar formulae with fat has become a technically easily-solved problem, with progressive approach to improved adaptation of cow's milk to human milk, at least in comparison to the early milk-water-sugar formulae.

SUPPLEMENTAL FEEDING

Technological progress has led also to the introduction on large scale of baby foods, different from milk formulae. Excellent products are available in form of baby foods covering items such as cereals, meats, liver, strained vegetables, fruits, etc. Their use is spreading widely in modern pediatric dietetics. In the United States at least 90 % of the infants are receiving supplemental food below three months, and often during the first weeks of life.

TABLE 4. *Osmolar Load and Water Balance*

Supply of Water				Expenditures of Water					Water in Excess of Expenditures	
	Preformed water*	Water of oxidation	Total water	Renal	Extra-renal at 21°C	Extrarenal at 34°C	Total at 21°C	Total at 34°C	At 21°C	At 34°C
Human milk	200	13	213	20	80	147	100	167	113	46
Cow's milk	200	13	213	87	80	147	167	234	46	−21
Cow's milk + carbohydrate†	200	13	213	61	80	147	141	208	72	5

* For concentration of 150 ml/100 cal (20 cal/oz). † Two-thirds of the calories from cow's milk and one-third from added carbohydrate.

One of the important contributing factors in the matter of early addition of supplemental food to an infant's diet is the widespread insistence of mothers. It should be conceded that vitamin D, ascorbic acid, iron and, at least in artificially-fed infants, the vitamin B complex are necessary dietary supplements. The argument, however, that semisolid foods may accelerate the anatomical and physiological development of the digestive tract is not supported by the study of normal growth. The first teeth, incisors, appear at the age of 5–6 months, a clear indication that the infant is not dependent on mastication before the end of the first half year of life.

Early spoon-feeding elicits the atavistic 'thrust' or 'extrusion' reflex, innate to normal infants. Through conditioning, in form of protracted spoon-feeding, this reflex, which is probably the expression of a natural defense reaction, may be suppressed. This might lead to frustration, to mental insult, the late effects of which might manifest themselves in neurotic reactions. Human milk and/or proper cow's milk formulae, with supplements of vitamins and iron, will cover all dietary requirements of the young infant—at least up to 4–6 months of life.

MATURATION OF INFANTS

The newborn, especially the prematurely-born infant, is at birth not fully endowed with all metabolic enzymatic equipment which characterizes later age periods. The renal function reaches its maturity at the age of four, perhaps even only 6 months. In its immature state, it shows impairment of concentrating power. In consequence, it handles with difficulty abnormal loads as represented by electrolytes and metabolic products of protein.

Cow's milk, with its high content of salt and protein, will be handled with difficulty in concentrated form, as will any other form of food with higher content of salts and protein than the more dilute foods.

Table 4 is from a statement of the Committee on Nutrition of the American Academy of Pediatrics (33). In the last column the figures for 'Water in Excess of Expenditures' show a satisfactory positive balance for human milk even at high environmental temperature. In contrast, the corresponding figures for undiluted cow's milk are negative and for the usual cow's milk-water-sugar formulae uncomfortably close to the zero-line. This is the reason why with high environmental temperature or in the presence of fever, supplement of water is recommended for infants fed such cow's milk formulae. However, the fact remains that it must represent a stress for the infant. Even with added water, the metabolic stress is demonstrable by the changed homeostasis, i.e., high figures for BUN, inorganic P and reduced Ca in the serum of young infants fed concentrated cow's milk formulae (cf 32). The high figures for BUN may be observed in infants fed milk formulae with high protein-content up to the 6th month of life (34).

Although urea may be excreted in the urine even of young infants (35) without significant increase of the obligatory urine volume, which is more closely regulated by electrolytes (36), this does not exclude the harmful effect of high protein intake on metabolic processes including water balance. On excessively high intake of protein (8–12 grams per kg and day) and calories (150–200 cal per kg and day) in premature infants growth and food efficiency ratio were significantly reduced (34).

To discuss further the immaturity of the newborn infant, more and more data are accumulating to testify to the immaturity of the young infant, especially of premature infants. About twenty years ago, Levine, Marples and Gordon found in premature infants, fed their recommended high protein formula, that they excreted in the urine high amounts of parahydroxyphenyllactic and parahydroxyphenlpyruvic acid. They also reported that with the addition of ascorbic acid this excretion has been reduced practically to zero. They related this finding to a disturbance or immaturity of the tyrosine oxidase system (37, 38). Kretchmer and his associates found that the activity of phenylalanine-hydroxylase is negligible in the livers of rats late in gestation, and in the early neonatal period (39–41).

Even more interesting are studies on the immaturity of red blood cells in newborn infants especially in prematures. Recently, Gross and Hurwitz (42) have found high values for glucose-6-phosphate dehydrogenase, aldolase and 6-phosphogluconic dehydrogenase. The increased metabolic activity of red blood cells in the newborn, and for a longer period of time in pre-

matures, may be linked to glycolysis and to the 'pentose-shunt', with gradual decline to values found for adult subjects in the second 6 months of life. The reduced glutathione is not lowered in amount but it is unstable in the red blood cells of newborn infants.

Of interest is the hemolysis of red blood cells by vitamin K. Analogues, such as Synkayvite caused occasionally hemolytic anemia in premature infants whose only medication has been prophylactic daily injection of Synkayvite in large doses (>10 mg/day) (43). Newborn infants (44) and again especially premature infants (45) have a physiological vitamin E deficiency detectable by the increased susceptibility of red blood cells—even *in vitro*—to H_2O_2. The same susceptibility of red blood cells—*in vivo* (46) and *in vitro* (47)—to Synkayvite and other vitamin K analogues may be demonstrated on red blood cells of vitamin E deficient rats. It may be assumed that these observations on animals are applicable to human newborn infants.

MILK SUBSTITUTES

In order to combat wide-spread protein-calorie malnutrition in many parts of the world with shortage of milk or other animal proteins, there is great need for substitute foods with high content of protein and adequate nutritional value. Such substitutes should serve in the first place as supplements to the existing meagre, unsatisfactory diet of toddlers, preschool children, lactating mothers, etc. However, the necessity may also arise that they should constitute practically the sole source of food for young infants. It would exceed the purpose of this presentation to review the voluminous literature on the whole subject. It should suffice to refer only to one interesting development pertaining to the use of soybeans in infant nutrition. Soya products have been widely used in East Asia, especially in China, for many centuries. In Indonesia, a fermented soya product called tempeh is still a very popular food. It is prepared in small factories and also in homes with the use of the fermentative fungus *Rhizopus oryzae* (48). In our own study with tempeh (49) we found it, when properly prepared, of distinctly higher biological value than unfermented, heated control soybeans, reaching the biological value even of skimmed milk protein. Further, we were able to identify[2] in tempeh the presence of a highly active antioxidant which adds considerably to the preservation of tempeh as food, and in biological test may act as analogy to vitamin E.

In the foregoing, several points were selected for illustration of the present state and possible future orientation of infant nutrition. It is safe to conclude that the last chapter has not yet been written in the history of infant nutrition.

[2] In cooperation with Dr. Kiku Murata (Osaka) in our laboratory.

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Nutrition and lactation

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IN ANY CONSIDERATION of the problems of maternal and infant nutrition the subject of lactation is obviously of prime importance. In most underdeveloped countries, women breast-feed their infants for prolonged periods. From the infant's point of view, the adequacy of breast milk to meet the nutritional demands, wholly or partly, in the different stages of lactation requires consideration. From the point of view of the mother, the interrelationship, if any, between her nutritional status and lactation has to be studied, and the optimal maternal nutritional requirements during lactation determined.

Studies of these aspects of lactation in poor Indian communities have been carried out by the authors and their colleagues. The results of these studies are briefly reviewed here. Though these observations pertain to poor Indian communities only, they may be considered to be of interest and significance to those engaged in the study of the problems of malnutrition in all underdeveloped regions of the world.

THE MOTHERS

The studies on lactation which are being reviewed here were conducted on Indian women belonging to the poor socio-economic groups. It will be pertinent here to give an account of the breast-feeding practices, dietary intake and nutritional status of these mothers.

Breast feeding and weaning practices. It was found that in poor Indian communities, infants were invariably fed at breast for prolonged periods. Breast feeding was usually started within 2 or 3 days after delivery and was continued into the second and even the third year of life. In a survey covering different parts of South India, Someswara Rao and colleagues (29) found that among poor communities 92% of infants were breast-fed at the age of 6 months; and over 50% at the age of 18 months. One child in every 5 was at the breast beyond 2 years of age while 1 in 8 was still breast-fed even at the age of 2½ years. However 98% of children were fully weaned by the age of 3 years. In a study of the hill tribes of South India, Bhavani Belavady and co-workers (7) found that the majority of mothers continued breast feeding till at least the end of the second year. In a large majority of the tribal children, complete weaning took place around the third year. Weaning was mostly determined by the onset of next pregnancy and not by any definite ideas or traditional beliefs regarding the optimal time for weaning. Breast feeding was done on demand and not according to a schedule. The frequency of feeding depended upon whether the mother was staying at home or working.

Supplementary feeding was started around 6 months in most areas. An important exception to this was Hyderabad in South India where the great majority of infants did not receive any supplementary foods till after the end of their first year (29, 32). Important differences in the picture of malnutrition in childhood between Hyderabad and other areas, possibly attributable to this factor, will be discussed later.

Diets. Diet surveys were carried out in a series of poor Indian mothers in different stages of lactation by Kalpakam Shanker and Venkatachalam (unpublished) in Hyderabad and by Pasricha (24) in Coonoor. The results of these surveys have been set out in table 1 along with the recommended dietary allowances in lactation. It will be seen that the diets of the mothers investigated were inadequate in several respects. Over 4% of the mothers investigated did not include pulses in their dietaries except occasionally, and over 21% did not take green leafy vegetables except rarely. Nearly 63% took no milk or milk products, and 47% had no meat or eggs in their diets.

Body weights. The mean body weight of mothers of the low socioeconomic group in different stages of lactation was found to be 42.4 kg (30.9 kg to 59.6 kg) (Kalpakam Shankar and Venkatachalam—unpublished). There were no significant differences in body weight between mothers in the early stages of lactation and those in the later stages. The body weights of lactating women with only one infant were not significantly different from those with more than one infant. In the poor communities investigated, the progressive increase of body weight with advancing age reported in normal American women was not noticed.

Serial body weights of 14 poor Indian mothers were recorded from the time of delivery at 4-weekly intervals for nearly 90 weeks (13). Serial estimations of the output of milk in these mothers indicated that they were secreting between 400 and 600 ml of milk daily. It was noticed that in some of the mothers the body weights remained practically stationary throughout the period of study. In those that lost weight the loss ranged between 1.8 kg and 7.3 kg, and it took nearly a whole year for this loss to occur. These findings are interesting when the poor

TABLE 1. *Nutrient Intake of Lactating Women in Coonoor and Hyderabad with the Recommended Dietary Allowances*

Nutrients	Actual Intake		Recommended Allowances	
	Coonoor	Hyderabad	Nutrition Advisory Com. Ind. Council, Med. Res.	Nat. Res. Council, U. S. A.
Calories	1860	1890	2700	3000
Protein, g	43	47	112	100
Calcium, mg	299	180	2000	2000
Iron, mg	23	18	20–30	15
Vitamin A, IU		800	3000–4000	8000

diets of these mothers and their remarkably satisfactory lactation are considered. This aspect will be discussed later.

Body composition. The longitudinal studies of body weight reported above had thus indicated that the poor mothers subsisting on apparently inadequate diets, in spite of prolonged successful lactation, did not show any progressive striking decline in body weight. This might suggest that the expected loss of body weight in these mothers was masked to some exent by excessive hydration resulting in changes in body composition. To investigate this question, Venkatachalam and Gopalan (33) carried out estimations of total body water in 14 poor Indian mothers in various stages of lactation. The body water formed on an average 59.2% of the body weight with values ranging from 46.3% to 73.3%. In 5 of the 14 subjects the body water was over 60% of body weight and in 2 of them over 70%. There was no relationship between the stage of lactation on the one hand and the percentage of body water on the other. The mean value for the body water reported for normal female adults of European origin is 51% body weight—range from 45.6% to 59% (23).

The question arises as to whether this high percentage of body water observed in some lactating women is relative, being brought about by a reduction in body fat, or due to an absolute increase in body fluids. Serial determinations of body water in the same subjects might be expected to provide the answer to this question. If the hydration was only relative, it could not have contributed to the masking of the loss of body weight during lactation. The subject obviously requires further investigation.

It may not be out of place at this point to mention that in pregnant women belonging to the same poor communities it was found that the body water was 56.1%, before the 12th week of gestation, 66.4% between the 20th and 28th weeks of gestation and 70.9% after the 28th week (31). The interesting feature was that while the increase in body weight which could be expected from the increase in the absolute amount of body water between the 12th and 36th weeks of gestation in these women was about 10.9 kg, the actual increase in body weight was only around 6 kg. Since the mothers were in positive nitrogen balance (18) it appeared that concurrent with increase in body water there was also a loss of body fat in these mothers during pregnancy.

Plasma protein and haemoglobin levels. Venkatachalam and Kalpakam Shanker (unpublished) carried out determinations of plasma protein and haemoglobin levels in a series of poor Indian nursing mothers in Hyderabad. The plasma albumin level ranged from 2.6 g to 4.3 g% (mean 3.7 g %) and the haemoglobin levels from 9.0 g to 15.9 g % (mean 13.6 g %). Malaria and hookworm infestation were not serious problems in the population investigated in this study.

OUTPUT AND CHEMICAL COMPOSITION OF MILK

Output of milk. Someswara Rao *et al.* (29) carried out a cross sectional study of the output of breast milk in a series of 180 poor Indian mothers in different stages of lactation. A serial study of the output of breast milk right from the first week after delivery to nearly 2 years after delivery was carried out in 14 poor Indian mothers (11, 13). The study revealed that the mothers secreted between 450 and 600 g of milk in 24 hours till about the end of the first year; even around the 100th week of lactation the 24-hour milk output was about 150 g daily.

Among poor Indian communities several articles of diet and some drugs are believed to be galactogogues. These substances are actually administered to the mother, immediately after delivery to promote lactation and continued for some time thereafter in some cases with a view to maintain lactation. Three substances claimed to be galactogogues, namely garlic (*Allium sativum*) cotton seed (*Gossypium hirsutum*) and tamarind (*Tamarindus Indica*) were investigated (12). None of these substances was found to have any striking effect on milk output. It must however be stated that the mothers investigated were already secreting considerable quantities of milk, and it might be argued that under such circumstance the effect of any galactogogue may not be significant. Garlic has been shown to be a source of plant oestrogens, 1 kg of this substance supplying 4000 mouse units of oestrogen (8). While large doses of oestrogen are known to suppress lactation, small doses have been claimed to help lactation by preventing abnormal engorgement of the breasts due to oversecretion (28). It might be worthwhile to investigate this substance further in cases of lactation failures.

Comparative chemical composition. Studies of the chemical composition of human milk have been carried out by a number of workers in different countries (10, 15–17, 19–21, 26, 30, 34). Bhavani Belavady and Gopalan (5) and Bhavani Belavady (3, 4) have investigated the chemical composition of milk from over 300 poor Indian mothers. The salient findings from these latter studies are briefly reviewed below.

In table 2 the chemical composition of milk from poor Indian mothers, after the first month of lactation, has been compared with that reported from other parts of the world.

It will be seen that with regard to the proximate

TABLE 2. *Comparison of Chemical Composition of Human Milk*

Constituents	Indian (Belavady and Gopalan)	American (Macy)	Australian (Wardlaw and Dart)	British (Kon and Mawson) Reading data	Bantu (Walker, Arvidsson and Draper)	Chimbu (Venkatachalam)	New Hebrides (Peter)	Wuppertal (Gunther and Stanier)
T. solids, g%	12.12	12.9	13.74	13–13.9*	12.8	10.88*	10.08*	
Fat, g%	3.42	4.54	4.95	4.78	3.90	2.36	3.8	3.59
Lactose, g%	7.51	6.8	6.43	6.95	7.10	7.34	5.0	
Protein, g%	1.06	1.06	1.41	1.16	1.35	1.01	1.10	1.20
Mineral matter, g%	0.16	0.20	0.18		0.20	0.17	0.18	
Calcium, mg%	34.2	34.4		29.9	28.7		25.8	
Phosphorus, mg%	11.9	14.1		13.0			15.2	
Potassium, mg%	34.7	51.2						
Sodium, mg%	22.1	17.2						
Magnesium, mg%	2.6	3.5						
Vitamin A. IU/100 cc	70	201		153*				133*
Thiamine, γ/100 cc	15.4	14.2		18.3				19.4
Riboflavin, γ/100 cc	17.2	37.3		25.5				21.4
Vitamin C, mg/100 cc	2.6	5.2		3.2–4.0	2.9			3.2–4.3

* These figures have been calculated from the values reported by the authors.

principles, there was no striking difference between the values observed in the milk samples from poor Indian mothers and those reported from other parts of the world. With regard to the vitamins, however, the values obtained among the poor Indian mothers were considerably lower than those reported by other workers. It would thus appear that the poor nutritional status of the mothers is more readily reflected in the concentration of some vitamins than in that of the proximate principles in their milk. Studies of the effect of dietary supplementation on the chemical composition of milk, which will be referred to later, also appeared to support the above conclusion.

Effect of pregnancy. The changes in chemical composition of milk brought about by pregnancy were investigated by Bhavani Belavady and Gopalan (5) in 12 mothers. The stage of lactation in these mothers at the time of the study varied from 15 to 24 months. In 4 mothers serial studies of the composition of milk during pregnancy were carried out. A marked increase in protein content of breast milk in the later trimesters of pregnancy was a striking feature (table 3). Quantitative studies (13) had revealed a marked diminution in the output of breast milk during pregnancy. Thus the increased protein concentration may not be the result of an absolute increase in the protein content. On the other hand, it was significant that similar increase was not observed with regard to the other constituents and that with regard to calcium there was actually a fall in concentration.

This finding is of considerable practical significance and would indicate that even in advanced pregnancy breast milk, in spite of considerable diminution in quantity, could provide significant amounts of protein to the infant.

Effect of dietary supplementation. It had earlier been pointed out that the diets of mothers investigated here were inadequate according to recommended standards. It seemed worthwhile to determine to what extent the chemical composition of the milk could be improved by dietary supplementation with the appropriate nutrients.

The results of investigations of the effect of dietary protein supplementation had been reported earlier (12). It has been shown that an increase in the dietary protein from 61 to 99 g daily did not result in an appreciable increase in the total protein output in milk in 24 hours. This observation however does not rule out the possibility that protein supplementation may have an appreciable beneficial effect on protein content of milk in women subsisting on very low levels of protein.

While protein supplementation had no striking effect on the protein content of milk, it was found to exert an interesting effect on another nitrogenous constituent of the milk in poor Indian mothers. In an investigation of the nitrogenous constituents in breast milk of a series of 67 poor Indian mothers, Bhavani Belavady (3) had observed that the mean figures for creatine and creatinine nitrogen in the samples investigated were 3.4 and 2.3 mg/100 ml of milk respectively, as compared to values of 1.1 mg for each of these constituents reported by Macy (21). The effect of protein supplementation on the creatine and creatinine content of milk was therefore investigated in a fresh series of 8 cases by Bhavani Belavady, Venkatachalam and Gopalan (unpublished). It was found that when the dietary protein intake by these mothers was raised to 90 g daily the creatine concentration showed a significant reduction. The creatinine concentration however did not decrease (table 4). The creatine and creatinine content of serum of these mothers was not high even in the initial stage before supplementation. The significance of the abnormally high creatine concentration in milk in these undernourished mothers and the manner in which dietary protein supplementation helps to correct this abnormality require detailed investigation.

The effect of supplementation of the maternal diet with vitamin A, thiamine, ascorbic acid and calcium gluconate for periods ranging from 3 weeks to 9 months in a series of poor Indian mothers were investigated by

TABLE 3. *Composition of Milk from Pregnant Mothers*

Case number	Duration of lactation, months	Duration of pregnancy, weeks	Amount of milk expressed, cc	Specific gravity	Total solids, g %	Fat, g %	Protein, g %	Lactose, g %	Vitamin A, IU/ 100 cc	Vitamin B_1 γ/ 100 cc	Vitamin B_2 γ/ 100 cc	Vitamin C, mg/ 100 cc	Total ash, g %	Calcium, mg %	Phosphorus, mg %
1	24	5	28	1033.3	10.67	1.73	1.46	7.38	23			4.5	0.095	24.0	9.8
		6	16	1028.0	11.92	3.10	1.58		39	14.0		4.1		23.0	11.9
		10	21	1032.1	11.22	2.45	1.30	7.37	20	17.1		3.7	0.102	18.6	15.5
		13	15	1029.5	11.52	2.97	1.36		88	18.8		6.4			
		15	18	1029.5	13.15	4.25	1.43	7.35		18.8		6.4	0.117		
2	15	8	64	1029.8	11.75	3.38	1.98	7.03	63	14.9	10.3	3.6	0.158	22.7	16.5
3	24	15	28	1030.8	13.02	4.22	1.21	6.92	120	13.1	9.4	2.6	0.137	18.6	10.3
4	24	15	5		10.29		2.40					6.3	0.356	21.9	13.0
5	24	16	5		13.14		6.99			14.8		7.2	0.369	23.7	12.5
6	18	16	16		17.59		1.94			11.9	12.0	3.2			
		22	5		20.92		3.17					2.7			
7	24	18	49	1024.8	11.62	4.30	1.35	5.67	55	13.6	8.3	4.3	0.302	16.1	6.0
8	24	20	24	1025.9	11.93	4.47	1.69	5.44	98	9.5	12.5	4.8	0.326	16.0	6.5
9	23	20	1				3.23								
10	20	16	7				2.78								
11	17.5	20	22	1020.7	12.11	5.74	1.10	4.97	57	0.7	33.3	3.0	0.300	27.5	6.0
		27	13	1032.0	14.23	4.98	1.27	7.76				2.4	0.219	40.6	14.0
		32	1.5				2.70					2.0			
12	17	24	1.2				3.96					3.3			
		31	6				4.58					1.0			
Average				1028.8	13.01	3.78	2.37	6.65	63	13.4	14.3	4.0	0.226	23.0	11.1
Data for milk from mothers lactating for more than 18 months (mean)			17.0	1030.8	12.28	3.41	1.13	7.51	66	15.1	11.5	2.9	0.154	36.6	11.1

Bhavani Belavady and Gopalan (6). Supplementation of the maternal diet with vitamin A for periods extending up to 9 months did not seem to have a significant effect on the concentration of this vitamin in milk. The subjects investigated had low vitamin A concentrations in their milks to start with, and their usual dietary intake of either preformed vitamin A or its precursors was very low. Even the lowest dosage of vitamin A supplement employed in this study, viz—2000 IU was far above their daily intake. It may be argued that the effect of vitamin A supplementation on the concentration of vitamin A in the milk may not be felt till the depleted hepatic stores of vitamin A in these mothers are replenished.

Venkatachalam, Bhavani Belavady and Gopalan (unpublished) investigated the serum vitamin A levels and vitamin A concentration in milk in a series of poor Indian mothers. The average serum vitamin A concentration was about 90 IU which is somewhat lower than the values for normal women reported by other workers. There was no correlation between serum vitamin A levels and milk vitamin A levels in the subjects investigated.

TABLE 4. *Effect of Protein Supplementation on some Constituents in Milk*

Total N_2 (mg/100 ml milk)		Creatinine N_2 (mg/100 ml milk)		Creatine N_2 (mg/100 ml milk)		Serum	
Initial	Final	Initial	Final	Initial	Final	Creatinine (mg/100 ml)	Creatine (mg/100 ml)
148.4	161.0	2.8	3.1	4.7	2.3		
176.4	219.8	2.9	3.5	5.4	1.2		
208.6	139.3	2.9	4.1	4.7	2.8		
205.8	162.4	2.6	4.7	5.4	3.6		
140.0	183.4	4.8	3.1	3.6	2.3		
380.8	404.6	1.5	4.7	1.7	1.6	0.8	0.2
143.5	163.4	4.5	4.9	3.3	3.3	0.6	0.8
156.8	175.0	3.7	4.7	2.6	1.8	0.5	0.6
Av. 195.0	201.1	3.2	4.1	3.9	2.3		

Supplementation with thiamine brought about a significant increase in the concentration of the vitamin in milk. Supplementation with ascorbic acid also brought about an increase in the concentration of the vitamin in milk. Serial studies with changes in the dosage level indicated that the minimal level of supplementation necessary to achieve and maintain maximal concentration of the vitamin in the milk in these cases was about 200 mg daily. This is not far removed from the figure of about 140 mg which was arrived at by Kon and Mawson (20) from their study in one woman.

Supplementation with 30 grains of calcium gluconate daily (180 mg calcium) for 2 to 3 months brought about a fall in the concentration of calcium in milk from an initial value of 41.0 ± 3.53 mg/100 ml to 25.0 ± 0.97 mg/100 ml of milk. In an earlier investigation (5) it had been observed that a group of poor mothers who were in the regular habit of chewing betel leaves with slaked calcium oxide had a lower content of calcium in the milk as compared to a comparable group of mothers not addicted to this habit. The mechanism underlying the drop in calcium concentration brought about by dietary calcium supplementation requires elucidation.

There was no correlation between the haemoglobin levels of the mothers and the iron concentration of their milk samples. In a series of mothers with low haemoglobin levels the effect of iron therapy for 3 weeks was investigated (5). Haemoglobin levels and iron concentration of milk samples were determined before and after treatment in these cases. It was noticed that while there was an increase in haemoglobin level in all cases after treatment, the iron concentration in milk showed a significant drop in several cases. However, it was not certain whether this decrease in iron concentration reflected an absolute decrease in the iron content of the milk, as no parallel study of the output of milk before and after iron therapy was carried out.

ADEQUACY OF BREAST MILK FOR THE INFANT

The important question with regard to the infant is: how adequate is breast milk to meet the nutritional demands at various stages of lactation? A serial study of the growth of infants along with parallel estimations of the output and chemical composition of milk of the mothers has helped to provide some information on this point (11, 13). The serial changes in average body weight of a group of poor Indian infants were compared with the weight changes of normal American infants. It was seen that the actual body weights of the poor Indian infants were lower than those reported at the 50th percentile for American infants of corresponding ages. The two growth curves were however nearly parallel for the first 6 months, signifying that though the actual weights were different, the growth rates were not unduly divergent for the first 6 months. Since all these infants were solely breast fed for the first 6 months and received supplementary foods only beyond this period it may be concluded that breast milk was fairly adequate to meet the demands of the infants up to 6 months of age even in poor socio-economic groups. It may be argued that breast milk was not fully adequate even during this period considering that the actual body weights of the infants were lower than that of normal American infants.

However after the 6th month the divergence between the Indian and American growth curves became progressive and marked showing that supplementary feeding becomes essential after this period and that the supplementary foods which the Indian infants were receiving were highly inadequate.

Caloric intake. It was computed that the caloric intake of infants fed solely on breast milk for the first 6 months was between 350 and 400 calories daily. This ration provided over 100 calories per kg of body weight till around the 10th week of lactation; after the 10th week the caloric intake per kg showed progressive decline till around the 24th week the caloric intake was around 70 calories per kg. If an intake of 100 cal/kg/day is considered optimal for infants, breast milk alone should be considered insufficient to meet the full caloric demands of the infants even up to 6 months.

Protein intake of breast-fed infants. It was found that the adequacy of breast milk during the first 6 months could not be improved by dietary supplement of protein to the mother (11). Comparison of the growth of infants of mothers receiving milk supplements and control groups did not reveal any striking difference. Considerable caution is however necessary in the interpretation of this result. Most of the mothers investigated had already a dietary protein intake of about 60 g daily and a fairly satisfactory lactation performance. It may be physiologically impossible to augment significantly an already satisfactory performance. Supplementations with vitamins might have perhaps yielded more striking results, considering the fact that studies of the chemical composition of milk in these poor communties had revealed deficiencies in the vitamin content and not in the protein concentration.

From a knowledge of the output and chemical composition of the milk it was calculated (11) that the infants investigated here received between 1.5 to 2 g protein per kg body weight during the first 6 months. It was considered that 2 g per kg rather than 3 g per kg was probably the protein requirement during infancy. The expression of protein requirement on the body weight basis may however be fallacious when applied to undernourished communities.

Adequacy of breast milk after six months. It was clear that breast milk by itself was inadequate to meet the full nutritional demands of the infant beyond 6 months. A comparison between the picture of malnutrition among children in Hyderabad and Coonoor has thrown some interesting light on this question. The children in both centres received breast milk for nearly 18 months to 2 years, but while the children of Hyderabad subsisted solely on breast milk for nearly 18 months those of Coonoor received supplementary foods from their 6th month. There were no striking differences in the chemical composition of milk between the mothers of Hyderabad and Coonoor except that the vitamin A content of the milk of the former appeared to be somewhat lower. In a comparative study of the clinical features of kwashiorkor in both these regions, Venkatachalam and Gopalan (32) found that the body weights of cases of corresponding ages were significantly lower, the incidence of skin changes, anaemia and associated vitamin B complex deficiency signs was considerably higher, and the general clinical picture was more severe in the cases of kwashiorkor observed in Hyderabad than in those seen in Coonoor. In a study of vitamin A deficiency, Gopalan, Venkatachalam and Bhavani Belavady (14) observed that the proportion of cases of keratomalacia to Bitot spots was much greater in Hyderabad than in Coonoor, indicating vitamin A deficiency of a more severe degree in Hyderabad. This may be partly attributable to the later institution of supplementary feeding in Hyderabad and partly also to the somewhat lower vitamin A content of breast milk in poor women of Hyderabad.

MATERNAL NUTRITIONAL REQUIREMENTS

Various recommendations regarding maternal nutritional allowances during pregnancy and lactation have been made. It would however appear that these recom-

mendations have not necessarily been based on definite scientific evidence. All available evidence would indicate that the nutritional requirement in pregnancy is not merely the nutritional requirement of the mother in her normal state plus the nutritional requirement of the foetus. Similarly, the nutritional requirement of the lactating mother is not evidently just the requirement of the woman in her normal state plus the amount represented by the nutrients in the breast milk. Presumably, there are profound alterations in metabolic behaviour during pregnancy and lactation, which have not been fully understood. The information available on this subject, with regard to the lactating woman, is even scantier than that with regard to the pregnant woman.

In table 1 the actual intake of different nutrients by poor Indian mothers has been compared with the recommended allowances. It will be seen that the prevalent dietaries of these mothers fall far short of the recommended levels. The observations presented in the previous pages have however shown that in spite of such seemingly inadequate diets, the poor mothers studied here were able to produce milk of satisfactory quantity and fair quality (at least with regard to proximate principles) for prolonged periods. Such satisfactory lactation in the face of apparently inadequate diets raises the question as to whether the existing recommendations regarding maternal nutritional allowances during lactation require closer re-examination.

Caloric requirement. The Second Committee of the FAO on Caloric Requirements (1957) recommended that a nursing woman secreting 850 ml of milk yielding 600 calories be provided 1000 additional calories from food if the body stores are not to be depleted. The Committee felt that in practice 800 calories would be a reasonable additional allowance. In the absence of data for assessing the caloric efficiency of human milk production, the above recommendation could be considered only tentative.

Venkatachalam and Gopalan (33) determined the basal metabolism in 14 poor Indian nursing women. The nursing women investigated were found to expend more calories per unit body surface per hour (40.2 cal) that normal Indian women in whom an energy output of 30.9 to 32.1 calories per m^2 per hour was recorded (25). The excess energy output which is nearly 26% over the average basal metabolic rate of normal adult women (or approximately 250 to 300 cal per day) may be presumed to reflect the energy needs for milk production.

The increased calorie requirements in lactation may be considered to have two components: *a*) increased caloric requirement for actual secretion of milk and *b*) the increased calorie requirement represented by the actual calorie value of the milk secreted. The increment of basal metabolism noted in this study may be considered to reflect only the first component since the caloric value of milk secreted may not be reflected as an increase in oxygen consumption. The quantitative studies in lactation referred to earlier revealed an average daily output of 400 to 600 g of milk during the first 6 months of lactation. The caloric content of this quantity of milk is equivalent to 250 to 400 calories. On this basis it may be claimed that the increased calorie requirement consequent on lactation in these subjects was of the order of 500 to 700 calories per day.

The actual dietary caloric intake in these mothers investigated here was between 1800 to 1900 calories. The basal caloric requirement (on the basis of the basal metabolic determination) plus the caloric content of milk in these subjects would work out to 1550 to 1700 calories. Thus the diet provided only 150 to 200 calories over and above the calculated minimum basal and lactation requirements in these mothers.

Protein requirement. The Nutrition Advisory Committee of the Indian Council of Medical Research (1944) had recommended an allowance of 100 g protein daily for pregnant women and 112 g protein during lactation for a woman weighing about 45 kg. There are similar recommendations from the nutritional organizations of other countries.

The assessment of protein requirement during lactation can be attempted in two ways. Firstly, the minimum level of dietary protein necessary to ensure maximal protein content of breast milk may be investigated. Secondly, nitrogen balance studies on different levels of dietary protein in lactating mothers may be carried out. The interpretation of the results of these studies would present difficulties but they may be expected to provide some information bearing on the problem.

It had earlier been shown that raising the level of dietary protein from 60 g daily to 99 g daily was not attended with any significant increase in the protein content of milk in these poor mothers. It was however shown that the abnormally high level of creatine observed in the milk in these mothers was reduced when the level of protein in their diets was raised to about 90 g daily. Though the actual significance of the high creatine content of the milk of these mothers is still a moot question, the fact that this abnormal trend could be corrected by raising the level of dietary protein would seem to be significant. A dietary protein level which could maintain the creatine and creatinine concentration in milk within normal limits may well represent the optimal protein requirement. This, however, is an aspect which requires further investigation.

Narasinga Rao, Pasricha and Gopalan (22) carried out nitrogen balance determinations at three levels of protein intakes in a group of poor Indian nursing mothers. It was believed that this study might provide information regarding the actual protein requirements of the women under their prevalent conditions.

It was found that the women were in nitrogen balance on an intake of 0.24 g/kg body weight/day, which was much above the reported minimum of 0.087 g nitrogen/kg body weight/day required for nitrogen balance in normal Indian adults (27). Thus the minimum protein needs during lactation in the mothers studied may be considered to be about 1.5 g protein/day/kg body weight.

Apart from elevation of the minimum level of nitrogen needed for attainment of balance, it was found that nitrogen retention in these mothers increased as the

protein intake was raised. This latter phenomenon might not be a specific attribute of the lactation state but could be the result of undernutrition. Beattie *et al.* (1) and Benditt *et al.* (2) had shown that in undernutrition there was a linear relationship between nitrogen retention and nitrogen intake. The dietary protein level which results in maximal nitrogen retention might be a criterion for computing the optimal requirement of the malnourished nursing mothers. In the mothers investigated here, it was found that an increase of intake from 60 to 100 g protein daily resulted in increased retention, but when the protein intake was further raised to 114 g there was no appreciable further increase in retention. Using this criterion, an intake of protein 100 g per day derived from the type of diets used in this study may be considered necessary for lactating mothers of poor communities.

The Committee on Protein Requirement of the FAO (1957) had suggested that about 2.0 g of food protein of high nutritive value may be needed to produce 1.0 g of milk protein. On this basis it can be calculated that the women studied here would not need more than 15 g over and above their usual requirements to meet the demands of lactation. It must be remembered however that the FAO recommendation applies to women who have been on an adequate diet in their normal state and throughout their pregnancy. The precise criterion which must be employed for the assessment of protein requirements in under nourished communities requires further investigation.

Vitamins and minerals. The minimal dietary levels of different vitamins and minerals needed to maintain their maximal concentration in the milk may be used as the criterion for the assessment of the requirements of these vitamins and minerals. While supplementation with vitamin B_1 and vitamin C was reflected in increased concentration in milk, this was not the case with vitamin A, calcium and iron. Thus while these supplementation studies may be used for assessing the requirement of some vitamins and minerals, they may not be applicable for certain others like vitamin A, calcium and iron. The effects of supplementation of vitamins and minerals on the chemical composition of milk have been reviewed in the preceding pages. The studies had indicated that an intake of 200 mg of ascorbic acid daily was necessary to ensure maximal concentration of this vitamin in milk. Similar studies with other vitamins now in progress may be expected to throw further light on the problem.

In all computations of the nutritional requirements in lactation, the hormonal factors governing lactation and their possible effect on conditioning nutritional requirement should receive important consideration. It has been shown that among the pituitary hormones concerned in galactopoiesis in cows the growth hormone may play an important part (9). The profound protein anabolic action of this hormone may be expected to influence the protein requirement. The effect of hormonal changes in lactation in conditioning nutritional requirement is an aspect that seems to have hitherto received scant attention.

CONCLUSION

In conclusion the following main problems with regard to lactation in underdeveloped countries would seem to require further investigation.

Extensive field surveys have indicated that there is almost an inverse relationship between the position of the mother in the socio-economic scale and her lactation. While it is tempting to suggest that this phenomenon is mainly attributable to emotional and psychological factors, careful examination would seem to indicate that this may not be the entire explanation. While a great deal of attention has been focused on the possible effects of undernutrition on lactation, not much thought has been given to the possible role of overnutrition in inhibiting lactation. This is an aspect which would seem to merit closer study. Field studies also suggest that the resumption of menstruation after delivery takes place after a much longer interval in the poor mothers than the well-to-do mothers. The implications of this finding with reference to lactation requires investigation.

The studies reported above have shown that supplementation of the mother's diet with certain vitamins would be reflected in increased concentrations of these vitamins in the milk. The possibility of improving the growth of infants in poor communities by improving the dietaries of the nursing mothers is a subject which would require further investigation under field conditions.

A great deal of further work seems necessary to elucidate the maternal nutritional requirements during lactation. The criteria for assessing the nutritional requirements require careful consideration, and the existing recommendations may have to be subjected to close scientific scrutiny. In such a consideration, the possible role of the hormonal factors governing lactation in conditioning nutritional requirement should receive adequate attention.

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Cultural and anthropological factors in infant and maternal nutrition

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THE CUSTOMS, practices and attitudes making up the local 'culture pattern' have a profound effect on the closely interrelated nutrition of the mother and child, and this is especially so among the poorer segments of the population in many technically developing tropical countries (2).

It is often helpful to divide customary practices into 4 categories according to their apparent public health value, as judged by unprejudiced analysis in relation to the local ecology, including the geography, climate, communications, range of foods available, and the human and animal disease pattern, and on imperfect present-day scientific knowledge (2). These 4 categories comprise:

1) *Beneficial practices*, such as prolonged breast feeding for tropical village infants. These should be supported and adopted into local health teaching.

2) *Neutral practices*, such as massaging the limbs with oil 'to make the bones strong' as in parts of India. These, which appear to have no significant scientific value or otherwise, should be left alone.

3) *Unclassifiable practices*, such as pre-chewing of foods by mothers in some communities and 'tongue-feeding' to their infants. These, for which different interpretations are possible, should also be left alone pending further observation and consideration.

4) *Harmful practices*, such as the failure to give young children fish in Malaya, where it is the main source of protein, because it is considered to produce worms. These are the main source of concern to the health worker and will require alteration by friendly persuasion and convincing demonstration—that is by health education—or by 'cultural integration' so that the ill-effects of a particular custom are modified while, at the same time, the essence of the culturally accepted practice is retained (3).

AREAS OF IMPORTANCE

While all aspects of a culture pattern are of importance and, indeed, can only be artificially dismembered, the following are areas which are always of especial significance in relation to the nutrition of the mother and her infant: 1) *pregnancy and childbirth;* 2) *food classification;* 3) *ideas as to disease etiology*, including methods of prevention and cure; 4) *methods of child rearing.*

CULTURAL FACTORS AMONG THE BAGANDA

The Baganda, a Bantu people, form the biggest and most 'advanced' tribe in Uganda, where their 1.4 million constitute one-quarter of the total population; 44% of the population of the country are children and it is they who bear the brunt of nutritional illness. Half the admissions to hospital are children, mostly under the age of 3 years, and kwashiorkor is the second commonest diagnosis, accounting for 10% of pediatric admissions.

Most families in Buganda live in rural areas each on their own plot (*shamba*), where the food crops of plantain, sweet potato, cassava, beans and groundnuts are cultivated by the women, and the cash crops of coffee and cotton by the men. The average peasant is poor and has had but little education, there being as yet insufficient schools. Medical facilities are quite inadequate for the needs of the people—for example, there is only one doctor per 20,000 of the population.

It is against this background of tribal society in a rural setting with minimal socio-medical facilities that customs and attitudes assume great significance in both the causation and prevention of malnutrition.

Selected examples of practices found in the culture pattern of the Baganda people of Uganda can be considered in relation to their probable category and to their etiological significance in the development of malnutrition in the mother or child, especially kwashiorkor. It may be noted that these customs will be of greater importance among the section of the population least exposed to modern education, do not apply uniformly all over the region and, in many instances, are in the process of spontaneous modification in the rapidly changing cultural kaleidoscope that is modern Africa.

1) *Pregnancy and Childbirth*

a) Food restrictions. Apart from the foods prohibited for all women, certain items are specifically restricted during

pregnancy. Most of these can be classified as 'neutral', including the avoidance of such minor, uncommon and nutritionally less important parts of the local diet as white ants (*nswa*), the heads of fish and sheep, baked plantain (*gonja*), certain types of yam and hot food (in the temperature sense).

Salt must be avoided or the newborn baby will develop *ennoga*, a culturally defined disease, probably made up of several skin conditions. This practice falls in the 'unclassifiable' group. It is perhaps useful in avoiding an excessive salt intake in pregnancy, with its added risk of toxaemia, although, on the contrary, it means that there will be an avoidance of the protein-rich part of the diet, the sauce (often of groundnuts) which contains salt, as opposed to the unsalted largely carbohydrate staple—steamed plantain (*matoke*).

Finally, a harmful practice, especially if carried to extremes, is a general restriction of diet in the attempt to avoid a large foetus and difficult labour.

b) *Clay earths.* A variety of clay earths (*emumbwa*) are traditionally eaten during pregnancy, intermixed with various chopped leaves believed to have medicinal properties. This practice cannot be classified, as the composition of the various earths is not known. It is possible that they may be sources of calcium, iron and other minerals.

c) *Drainage of the placenta.* It is customary for women to deliver in the kneeling position, and, after the baby is born, he will be left on a clean plantain leaf between the mother's legs until the placenta is expelled. This practice is probably beneficial in most cases, because, as the neonate is at a lower level than the placenta, maximal drainage of blood will occur, representing a valuable addition to the baby's iron stores.

2) *Food Classifications*

a) *Clan foods.* Each of the clans, of which the Baganda people are composed, has a totem, which may not be eaten by a clan member. These restrictions, which include grasshoppers (*nsenene*), lungfish and one variety of bean, can be regarded as 'neutral', in that the item restricted is never a major or common food.

b) *Foods prohibited for women.* As in several parts of East Africa, eggs and chicken are proscribed, as are mutton and several species of fish. This is entirely harmful, assisting materially in the development of protein sub-nutrition in women, which is of especial significance in pregnancy and lactation.

c) *Foods in childhood.* Two fruits are thought to be especially 'children's food'—pawpaw and passion fruit, which is plainly a minor beneficial attitude. Unfortunately, there is no traditional emphasis on the need for high protein foods especially prepared for infants. In fact, there are some who hold the view that certain valuable protein foods are unsuitable for children. Thus, eggs (if given before the teeth have erupted) lead to stupidity, fish will produce a rash, and meat make the child greedy; while white ants (*nswa*) and beans lead to diarrhoea.

Unfortunate recently introduced 'neo-nutritional' harmful customs (4), which also have a direct relevance to protein-calorie malnutrition, are the alleged value of tea for infants and the increasing prestige value of tinned powered milk, bought in very small amounts and fed in homeopathic dilution from an unclean bottle, often resulting in the disastrous vicious circle of infective diarrhoea—malnutrition (5).

d) *Significance of matoke.* Probably one of the major harmful dietary attitudes in the feeding of young children is the overemphasis on the value of steamed plantain (*matoke*) as food, as opposed to other items of the diet which are regarded as insignificant.

While this is certainly unfortunate nutritionally, as *matoke* contains only 1% protein, it is necessary to realize the very great emotional and symbolic part the plantain plays in the local culture pattern. Not only is the legendary introduction of the plantain to the country considered to have been made by Kintu, the founder of the Baganda, but, in the present day, the plantain plays a role in every aspect of life. Beer (*pombe*) is fermented plantain. The placenta is buried under a plantain tree. Food is steamed in plantain leaves. The baby is born onto a fresh leaf and a shroud can be made of old leaves. The significance of the plantain, both as a symbol of the Baganda people and of general 'goodness', is emphasized by the fact that the badge of the Women's Clubs has a plantain tree on it; while when the traditional ruler, the Kabaka, attends a special function the roads along which he will pass are lined with freshly planted plantain shoots.

It is then apparent that, in this cultural setting, it is unlikely that health education attempting to persuade mothers that *matoke* is a poor food for their young children will have any success. It is more likely that 'cultural

FIG. 1. Plantains being peeled by Muganda woman for preparation of *matoke*.

integration' may be achieved by teaching that *matoke* is a good food as a source of energy, but is even better when given with other items, such as legumes.

3) Ideas as to Disease Etiology

Cases of kwashiorkor of different stages of severity and with the main emphasis on different signs are called by a number of names:

a) Obwosi. In this the young child becomes weak, miserable and stops crawling. It is recognized that this occurs when the child is displaced from the breast by a younger sibling and often sent away to a relative.

b) Omusana. These cases—scientifically comprising a mixture of children with mild kwashiorkor and others with hookworm anaemia—are recognized by the light-coloured hair and pallor of the skin. The condition is thought to be due to the effect of the sun's rays.

c) Empewo. This word, meaning 'cold' in Luganda, is used because affected children have cold oedematous feet and also because they are thought to have been affected by cold when put out of the mother's bed to avoid the 'heat' of pregnancy.

d) Obusoro. Diagnosis is based largely on the finding of a very distended abdomen. The condition is considered to be due to certain "little animals" getting into the child's body. Treatment consists of the child being gently beaten with twigs of the same plant so that seeds are scattered about, and the 'cause' of the disease symbolically driven out.

These attitudes are essentially harmful, as, in common with many parts of the world, the malnutrition actually responsible is not recognized, although the situational diagnosis implied by *obwosi* shows the same understanding of the social etiology as did the word 'kwashiorkor' itself in the Ga language.

4) Child Rearing

The main aspect of child rearing of importance in the etiology of kwashiorkor is the practice of sending the young child away to a relative on the arrival of a younger sibling.

It seems likely that this is still practised by the majority of parents. Reasons for doing so were more tangible in the past when this was recognized as a way of raising the status of the child by sending him to an eminent relative and ensuring that he would inherit within the clan. The reason nowadays is largely that it has persisted as a local custom, while probably rationalized explanations are that it is to comfort an elderly relative; it cements clan solidarity, avoids spoiling the child, ensures schooling, enables the child to benefit from a relative's knowledge of medicinal herbs or allows the mother to resume her cultivation without having two small babies to look after. Conversely, according to some, not to send a child implies a distrust of relatives.

The sympathetic observer may agree with some of these reasons, while, at the same time, realizing that the practice is nutritionally harmful. Not only is the child suddenly cut off from the probably small amount of breast milk he may be receiving but, in addition, it has been shown that the psychological trauma of this abrupt geographical separation results in the development of an acute maternal deprivation syndrome in which anorexia is a marked symptom (1).

CONCLUSION

A knowledge of the culture pattern is essential to a full understanding of the factors underlying the local disease picture. More important, the division of customs into the various categories suggested permits the health worker to proceed within the framework of people's ideas, attitudes and beliefs, actively supporting beneficial practices and modifying or dissuading away from those considered on mature judgment to be harmful.

The bare bones of scientific fact function more certainly and harmoniously when clothed by the muscles and sinews of the familiar and trusted local culture pattern.

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Digestion in infancy

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THE WORD 'INFANCY' means 'early stage of development'; 'digestion' may be defined as 'a process by which complex ingested materials are reduced in the alimentary canal to simpler substances which can be absorbed into the blood and lymph.' Presumably the phase of digestion may be considered as ended when absorption takes place, although it is difficult to be dogmatic on this point since it is sometimes stated in respect of certain substances, e.g. dipeptides and disaccharides, that digestion is completed after absorption. An intimate study of digestion entails a knowledge of *a*) events in the passage of ingested food along the alimentary canal; *b*) the mechanisms, neural and humoral, by which its passage is coordinated; *c*) the enzymes, in glandular secretions and derived from the desquamated epithelial lining of the alimentary canal; *d*) the effects on absorption of digestants—agents in the ingesta, e.g. ascorbic acid and vitamin D, in secretions, e.g. bile salts—and of digestants and interfering substances in the ingesta or in the products of digestion; and *e*) the contribution of microorganisms in the gut to the breakdown of food. Knowledge is also needed of the form in which components of the food or their breakdown products are absorbed.

It is manifestly impossible to review the subject completely in the space allowed for the paper and the time allotted for the presentation. A general knowledge of the subject will therefore be assumed, such as is found for example in chapters 8 and 9 of the account by Clement A. Smith (57). I shall be concerned with points which are to me of special interest and I shall include a bibliography containing references to illustrative investigations and to reviews and reports of symposia. It is appropriate to my contribution to this Symposium on Maternal and Infant Feeding to refer to the relationship between the 2 subjects, particularly on the effect of the nutrition of the mother *a*) on the development of the infant's digestive capacity during intra-uterine life and *b*) on the mother's milk in relation to digestion in infancy.

TECHNIQUES OF INVESTIGATION

Many techniques are employed in the study of digestion, the results of almost all of which are difficult to interpret. The effect of the food consumed on growth and development may be studied; this of course not only includes digestion, but also the role of the absorptive system, utilisation, and dietary defects—level and balance of nutrients in the diet. Work on many factors which affect protein digestion and the absorption of the products of digestion in the intact animal has recently been reviewed and "the limited data available on the passage of dietary protein, or its digestive products, suggest that a number of dietary factors may have an influence on the rate of passage. The effects of total food intake, the effects of various dietary components, and, possibly, the effect of age of the intact animal should be considered as important areas for investigation" (41). The results obtained in a study of the digestion of starch (12) illustrate the changes brought about by the microflora of the gut. It is interesting in this context to note that 'coefficient of absorption' and 'coefficient of digestibility' are regarded as being synomymous terms!

Attempts are made to make allowances for some of these complicating factors in measurements of 'true digestibility'. Measurements of 'apparent digestibility' employing inert substances, e.g. chromic oxide (14, 44) admixed with the food, are useful.

Interest in techniques for studying digestion and absorption has been stimulated by the recognition of the 'malabsorption syndromes' (33). These studies in relation to digestion in infancy direct attention to the knowledge which can accrue from a study of pathological disturbances, as, for example, congenital absence of digestive enzymes and congenital disease of the pancreas and disturbances which follow the introduction of mixed feeding, as in the form of coeliac disease which, it is suggested, should be renamed 'gluten-induced enteropathy'.

Some light may be thrown on the digestive process by studies made on the occurrence of products of digestion in the blood and lymph draining the alimentary canal; the results of such studies also include the effect of the absorptive process and changes in concentration in the body fluids and is complicated by factors affecting utilisation. Experimental procedures have been designed, e.g. exteriorisation of loops and fistulae of the gut, to assist in the interpretation of results based on the levels of products of digestion in the blood. Useful

information has also been obtained from work done *in vitro* on sacs of everted small intestine (40).

Another approach to the study of digestion is by examination of blood and lymph for substances, introduced in the food, having properties by which they can be recognised after absorption (see next section for absorption of antigens and antibodies). A good example of this approach is the observation made by McCance and Brown (30) that an esterase is only found in a puppy's blood when it has been fed on bitch's milk which contains that esterase.

It is suggested that more research might be done on the digestion and absorption of what might be called 'biologically marked' constituents of food. There are several examples in the literature of the use of isotopically marked substances (15), e.g. I^{131} in the study of digestion in children with and without cystic fibrosis of the pancreas (27) and in young rats (1); N^{15}-labelled yeast protein and hydrolysates (11); the use of tritium-marked material in a study of the contribution of impaired digestibility to the reduced protein value of heat damaged pork (13).

Much of our knowledge has come from studies made *in vitro* of the effect of a succession of enzymes on foods and their constituents. Again the interpretation of results presents special problems; the value of this type of study will depend on the accuracy of the information of conditions obtaining in the various parts of the alimentary canal.

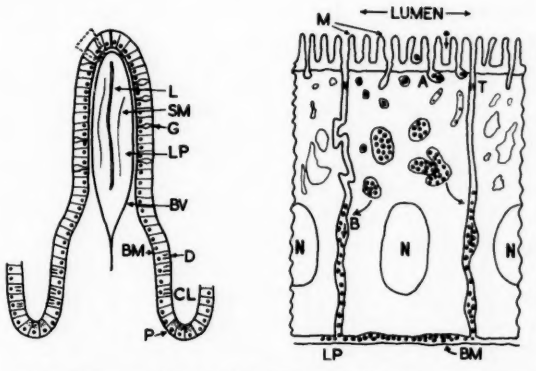

FIG. 1. Diagram of villus (left) and of epithelial cell indicating route of absorption of particulate fat and protein: (i) villus shows mitosis in columnar epithelium at (D) within the crypt of Lieberkühn (CL); these cells slide along the basement membrane (BM) to be desquamated at the tip of the villus. Substances pass from lumen of gut through the epithelium to the lamina propria (LP) thence to the blood via the capillaries (BV) or to the lymph via the lacteals (L); G = goblet cells secreting mucus; P = Paneth cells; SM = smooth muscle fibres. (ii) (inset from villus) shows route of absorption of protein (in early infancy) and of fat. The particulate substance (•) passes between the microvilli (M) of the striated border and is taken into the cell (at A) by pinocytosis (= drinking, as distinct from phagocytosis = eating, by cells) or, less likely, by passing into membrane-lined tubules within the cytoplasm. Particles leave the supranuclear (N = nucleus) cytoplasm at B by a 'reversal of pinocytosis', then pass between the epithelial cells to the basement membrane; they cannot pass from the lumen to the intercellular spaces because of a terminal bar T, which encircles the cell.

FOOD DIGESTED IN INFANCY

The food which is digested by an infant mammal in the early days of extra-uterine life is its mother's milk. The early secretion from the mammary gland, the colostrum, has special properties, notably in respect of its antibody content. It has been shown that those animals which do not receive any antibodies before birth, or are partly dependent for receiving them on the colostral whey, absorb whole proteins. Brambell and his co-workers (4) have briefly summarised the observations on this subject (see also 18, 1, 31). A schematic representation of some of the steps in the absorption of whole proteins (9) and of fat (42, 43) is given in figure 1. "Substances absorbed from the lumen of the intestine must traverse at least nine successive phases in their journey from the lumen to the blood or lymph: (1) the apical surface and striated border of the epithelium, (2) the cytoplasm of the epithelium, (3) the basal or lateral surfaces of the epithelial cells, (4) the basement membrane underlying the epithelium, (5) the loose connective tissue of the lamina propria, (6) the basement membrane surrounding the capillaries and lacteals, (7) the outer surface of endothelial cells, (8) the cytoplasm of the endothelium, and (9) the inner surface of the endothelium" (42).

There is some uncertainty about the human subject in whom "transmission of passive immunity takes place mainly, and probably entirely, before birth . . . and . . . there is little evidence, and none that is very convincing, of the transmission of immunity to the infant by way of the mammary secretions" (4). The fact that absorption of antibodies ceases does not necessarily mean that the absorption of whole proteins no longer occurs. Small amounts of egg albumin (21) can be shown to enter the blood of normal infants and in increased amount in infants suffering from diarrhoea. Even the adult may absorb whole protein from the alimentary canal (7).

In the study of the pathways of absorption of fat by electron microscopy (43) evidence is presented in support of the theory of particulate absorption of fat; but it is not possible by this means to evaluate the relative importance of this pathway as compared with that requiring partial or complete hydrolysis of the fat. Holt (24) and Frontali (20) have recently reviewed the subject of absorption; no clear picture can be offered of this subject, least of all of the role of fatty acids on the digestion and absorption of other nutrients.

In recent years interest has been aroused in the digestion of young farm animals (calves, pigs and lambs) by the attempt to introduce rations to replace mother's milk. These studies have led to the discovery that the animal develops enzyme systems, notably for digesting starch and certain disaccharides, at about the same time that mixed feeding naturally begins (61, 62). It is interesting that the columnar absorptive cells of the 18-day-old rat lose the ability to ingest proteins (9) and

that this is about the time at which the animal begins to 'creep feed' (at about 12 days (38)).

From a number of recent reports (48, 10, 2, 32) it appears that around 3 months is the time at which mixed feeding may begin in the human infant and it is of interest, in view of the comparative difficulty with which the infant digests starch, that this is about the age at which the baby begins to drool. There is some old evidence (52) that an infant has a greater ability to digest starch if it has been habituated to eating it. Perhaps the most interesting observation that emerges from the surveys of mixed feeding of human youngsters is the value of meat and meat products. In many parts of the world some breast milk may be given to young children up to the ages of 3–4 years or even longer (52, 46). I should like to conclude this section with a quotation from a paper I published in 1955 (49): "on the basis of the evidence available, I have wondered whether we should regard the mother during lactation not merely as a provider of food but also as a supplier of substances ready made for use by the infant until such time as he can synthesize them for himself. Is breast-feeding, therefore, a device by which provision is made for a transition between complete dependence on the mother before birth and complete independence after weaning from the breast?."

DIFFERENCES BETWEEN HUMAN AND COW'S MILK FOR FEEDING THE HUMAN INFANT

Some reference to this subject has been made in a symposium (55) and the biochemical differences between cow's milk and human milk for infant feeding have been reviewed several times in recent years (54, 50), with special reference to the differences in the properties of the casein in the 2 milks (35). In a recent review attention is given to some comparatively newly discovered constituents (37), notably sialic acid (neuraminic acid), of which there is twice as much in human as in bovine milk. In some as yet unpublished work we have recently found that the N.D.-p. value (53) of cow's milk expressed as Calories per cent total Calories is 10 as compared with the value, in the same terms, for human milk of 8. The protein:calorie imbalance (50) may be greater than these figures indicate. The ratio of total protein:lactose differs in human milk and cow's milk; if that in human milk is better suited to the nutrition of the human infant, then if, as seems likely, casein and whey proteins have different contributions to make to the body, the casein: lactose ratio of cow's milk may be even further removed from the optimum for the human infant, since "the casein content of breast milk is only about one-sixth of that of cow's milk, but the whey protein content, both relative and absolute, is higher" (37). Further, the absorption of calcium and other minerals is related to the amounts of phosphopeptides produced from casein (36). The calcium content of cow's milk is several times that of human milk and the absorption of calcium and possibly other minerals is likely to be high, perhaps excessively high, in artificial feeding.

There are other differences between the 2 milks, e.g. the essential fatty acid content of human milk is 3–4 times higher than it is in cow's milk. The significance of many of these differences is not yet understood and their relevance to the subject of digestion has as yet scarcely been considered. I think in examining the relative merits of human and cow's milk for the feeding of young children more attention should be paid to the possible effect of the 2 regimes on health in later life. For example, what are the sequelae, if any, of the gastric secretion which is 4 times as high in the infant fed on cow's milk as compared with that in the infant fed at the breast? (19).

THE ROLE OF THE STOMACH IN DIGESTION

Some observations first presented to the Physiological Society in 1951 (3) on the behaviour of milk in the stomach of the suckling rat may be summarised as follows (see also 47, 49):

a) Stomach contents of the suckling rat 1 hour after its last feed on its mother's milk consist of compressed curds arranged in concentric shells, strata or layers. The number of layers does not depend on the number of feeds; there may be 2 or more layers laid down at one feeding.

b) A drawing from a slice of the stomach contents taken longitudinally is shown in figure 2. By feeding the suckling alternately by a mother secreting coloured milk after injections of trypan blue and by a mother with uncoloured milk, it can be shown that the milk, as it enters the stomach, more or less completely envelops the residues from previous feeds.

c) With the use of this technique it has been shown that some curd may remain in the stomach for as long as 20 hours.

d) The whey fraction of a feed leaves the stomach relatively quickly; the diagram shows that almost ⅔ of the whey fraction may have left the stomach 40 min. after the feed (see fig. 3).

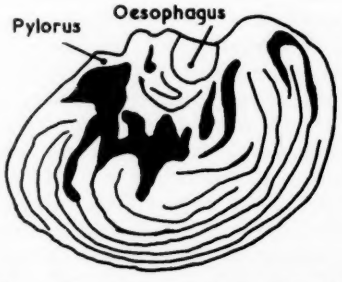

FIG. 2. Section of stomach contents (mother's milk) of 10-day old suckling rat; this appearance is obtained on leaving the section to stand for some hours in an incubator. The black lines and masses indicate a translucent appearance in the curd due to autolysis between successive 'shells' or layers of curd and in the pyloric mush.

e) Disintegration of the curd takes places in the antrum of the pylorus. This material we call 'pyloric mush.' It is often bile stained and has been described by Mayer as the 'amber contents' of the stomach (34). Reverse peristalsis from the duodenum to the stomach has been noted as a common phenomenon; bile, normally absent from gastric contents of adults, was present in about ¼ of the samples from either fasted or non-fasted infants (68). Any lactose remaining in the stomach is hydrolysed after about an hour into the component monosaccharides, galactose and glucose. (The products of digestion are probably drawn into the pyloric antrum, aided no doubt by pressure exerted by the muscle activity in the whole of the stomach. The emptying of the contents of the stomach may be determined mainly by the accumulation of fluid in the pyloric antrum (59).)

f) The stomach contents after a breast feed taken on an empty stomach show no stratification, much mucus and many air pockets; the normally stratified appearance throughout the clot takes 1–2 days to become re-established.

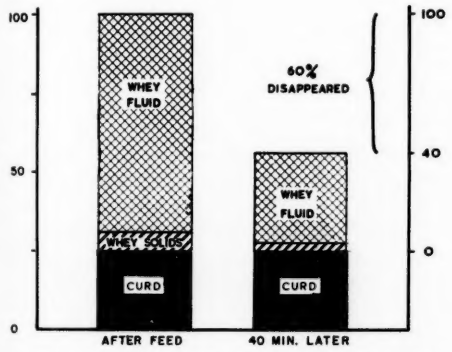

FIG. 3. Shows amounts of whey (fluid and solids) and curd in suckling rat's stomach immediately after a feed and in a similarly fed litter mate 40 minutes later; ordinate on left of chart relates to total stomach contents; the ordinate on right of chart indicates whey component.

For many years it has been known that stratification of food does occur in the stomach. The first observations are generally attributed to Grutzner (22); however, W. B. Cannon in 1898 described, in the first volume of the American Journal of Physiology, a stratified appearance, observed by radiology, of a cat's stomach after feeding alternately preparations with and without a radio-opaque substance (8). The arrangement of foods of successive feeds having different physical properties has been described by Scheunert (56). The outstanding difference between the layering that occurs after feeding successive portions of bread and the present observations on milk is that the order in which the layers appear is reversed. The last portion of bread paste given to the rat will be found in the centre of the stomach contents, whereas curd from the last feed of milk is always on the outside of the clot.

Investigators of gastric function almost invariably apply tests to an empty stomach. From observations on the rat, it would seem that the results of all such studies would be open to criticism as evidence of the behaviour of milk under natural conditions. Wiesener and Hirtzman have complained (66) that in spite of repeated efforts by numerous investigators it is still not possible to give a clear picture of normal protein digestion in the human infant; yet it appears from their own work (67) on the changes occurring in the stomach contents with time, and their observations on enzyme activity and clotting of both human milk and cow's milk preparations, that their practice was to wait 8 hours after a feed before giving a test meal.

It would be expected that emptying times of the stomach based on single feeds given on an empty stomach would be underestimated because, as Wolman has remarked in relation to the human infant, milk introduced into the stomach will discharge into the duodenum whilst it is still liquid in the first few minutes after drink-

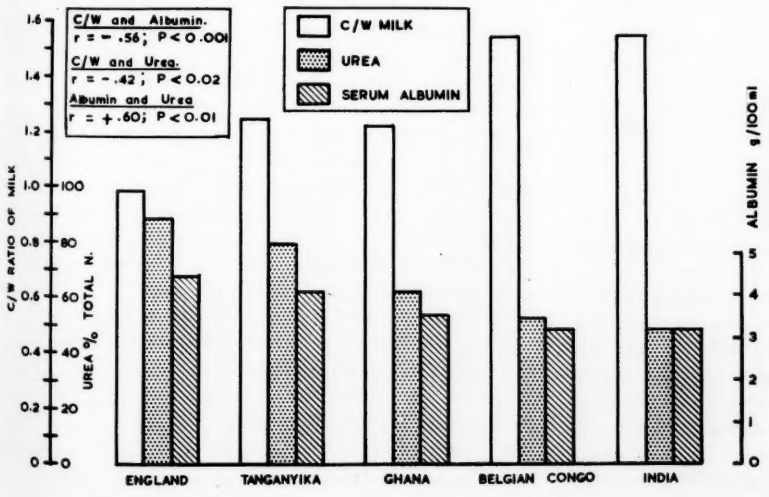

FIG. 4. Mean levels of milk curd/whey (C/W) ratio, urinary urea-total nitrogen and serum albumin for African and Indian mothers compared with values for normal English mothers.

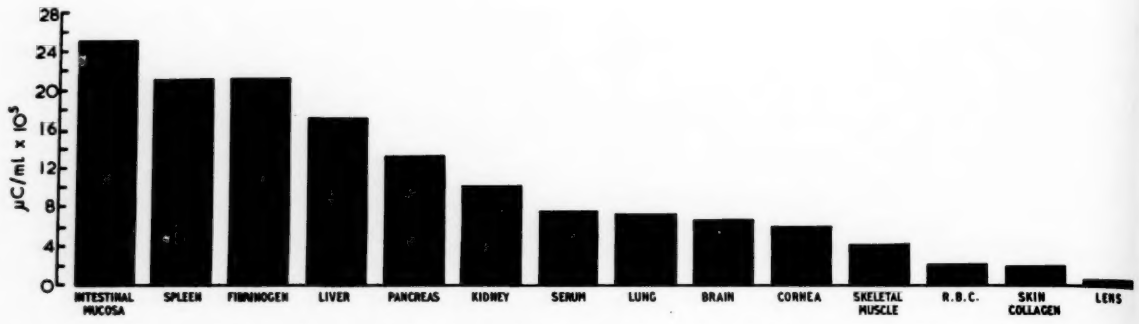

FIG. 5. Distribution of tritium in the proteins of a body of a young pig after administration of ^{3}H-DL-lysine, showing highest specific activity in intestinal mucosa.

ing (68). Smith (57) reviews evidence showing that in a considerable number of infants studied in various investigations, even after a single meal a considerable residue may remain in the stomach for 8 hours or longer and he refers to an investigation in which the second meal given 4 hours after a barium meal "caused portions of the first to remain somewhat longer in the stomach than if a second feeding was withheld pending complete departure of the foregoing one." The evidence in support of the view that "there is a big functional reserve to the various components of the digestive tract, and, other things being normal, gastric digestion is dispensable" (25) may apply to the human adult but I cannot agree that it applies to the human infant. I don't think, in view of the evidence available to him, that Smith (57) is justified in concluding that "gastric digestion (or digestive preparation) is not a factor of primary importance to nutrition at this time (early infancy) of life." Gastric emptying would seem to be a rate-determining factor in digestion. In animal husbandry the significance of the clot in the stomach for the successful nutrition of young farm animals is being increasingly appreciated (5) though no work has been done so far as I am aware on the contribution of stratification of the clot to its function. What appears to be emerging is the notion, in so far as proteins are concerned, that digestion is not merely a matter of speed of hydrolysis. Indeed, Mellander (36) has said of his work on the digestion of milks in the infant stomach: "the nutritional value of a protein does not depepd on its hydrolysing in the intestinal tract. The contrary might be true."

THE CURD:WHEY PROTEIN RATIO IN HUMAN MILK

Some evidence of differences in the behaviour of the curd and whey fractions of milk in the stomach of the infant rat has been presented above; some measurements are summarised in figure 3. These observations led us to investigate the curd:whey protein (C/W) ratio of milk from mothers in various states of protein nutrition. Some results of this study have been reported (29); these and other observations made in collaboration with Dr. C. R. C. Heard are summarised in figure 4.

It is known that the proteins of the milks of various animals are derived from extra-mammary sources (26) as well as being formed from amino acids and possibly also from peptides in the mammary gland itself. The full significance of these observations has not yet been determined but it is safe to conclude from them that the effect of malnutrition of the mother cannot readily be determined from an estimation of the total protein content of the milk.

MALNUTRITION AND DIGESTION IN INFANCY

Much can be learned about normal processes in the body from a study of relevant pathological processes, whether deliberately induced or presenting as a disease; this may well be true of conditions due to malnutrition. It may be profitable to examine briefly the relationship between malnutrition and the digestive process in the infant and young child.

It is possible that the full-term offspring of a malnourished mother is immature, i.e. although underweight and underdeveloped, it may not be premature. The veterinary nutritionist recognises the runt or reckling in a litter of pigs and knows that it has, compared with its litter mates, an impaired digestion. The work of Werner (65, 55) shows that the premature infant is not so well endowed at birth as is the normal infant with pepsinogen granules in the gastric mucosa or with zymogen granules in the pancreas. It may be that similar changes may occur in the immature human infant.

Derangement of gastric function has been known for many years to occur in infancy (16) and frequently after diseases due to infections, such disturbances may be analogous to dyschondrogenesis leading to arrest lines in bones which were originally associated with infectious episodes; these may have been due to protein deficiency since similar lines can readily be produced in animals suffering only from protein malnutrition (58).

Vomiting has been reported in protein malnutrition (60), particularly when unsuitable foods are given. There are reports in a recent review (64) of impaired gastric secretion in infant malnutrition; also many workers have reported reduced production and secretion of pancreatic enzymes in malnutrition in the young. Notwithstanding,

there seems to be no unanimity about the effects of these pathological changes on digestion in protein malnutrition, possibly because the test load imposed on the digestion is not unusually high for the malnourished subject. Werner (55), however, reports that she is attempting to apply tests to measure the capacity of the pancreas to continue to produce proteolytic enzymes and not merely to saturate the fluid produced during the basal secretion. Nevertheless there is a good deal of evidence that gastrointestinal disturbances, which are at least in part of nutritional origin, are a feature of protein malnutrition (63, 6). The projected investigations of the Cape Town workers on the value of amino acid mixtures as compared with the value of milk formulae and protein mixtures may help in the elucidation of the role of digestion and absorption. Mellander (36) envisages the possibility that "in the evaluation of vegetable protein combinations of high nutritional value to use as substitutes for animal protein, it is possible that not only the amino acid content has to be taken into consideration, but also the type of peptides formed physiologically. It is possible that the larger the peptides of a certain protein which an organism is able to utilize, the better the nutritional quality of the protein." A compensating mechanism has been suggested by Nasset (39) who says "the ingestion of protein stimulates the digestive tract to secrete endogenous protein which becomes mixed with food protein to provide an amino acid mixture that remains relatively constant in composition"; the operation of this mechanism in the protein malnourished does not appear to have been investigated.

Impressed with the almost universal occurrence of gastrointestinal disturbances in the marasmic type of protein malnutrition in infants, we have given special attention to the role of changes in the small intestine in the symptomology of protein malnutrition. That this is likely to be important can be inferred from the high rate of turnover of proteins in the mucosa of the intestinal tract; this is indicated in figure 5 from the specific activity data for proteins from piglets to which tritium has been administered (15).

Leblond and Stevens (28) have estimated the time for the renewal of the epithelial cells in the duodenum to be

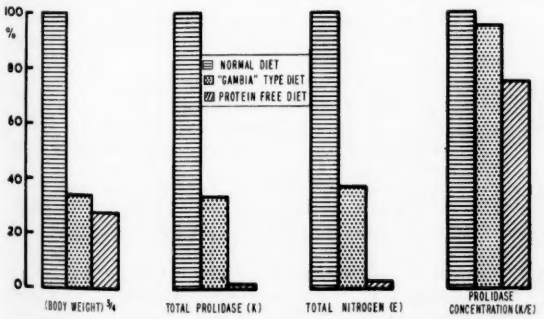

FIG. 6. Showing intestinal prolidase content in relation to body weight and total nitrogen for rats on normal, low protein and protein-free diets.

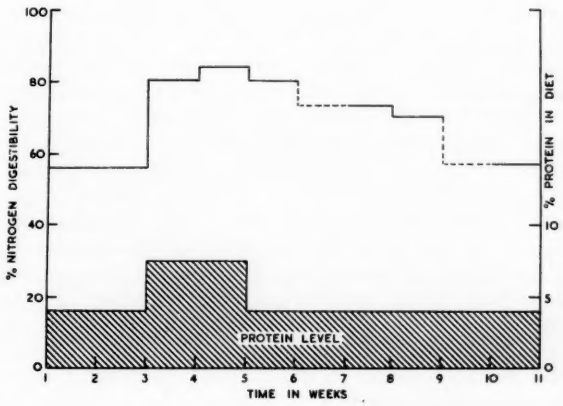

FIG. 7. Apparent digestibility of nitrogen in pig on low protein diet, showing an increase when percentage of protein in the diet is raised.

TABLE.* *Comparison of Faecal Nitrogen of Gambian Children With Others at Similar Levels of Intake*

		Nitrogen (g/24 hr.)		Apparent N digestibility %
		Intake	Faecal output	
Gambia children 2 to 5 years (5)	Range	2.36–4.90	1.10–2.22	53.3–75.1
	Mean	3.68	1.37	58.1
European marasmic infants (11)†	Range	2.39–4.83	0.41–0.86	74.0–89.9
	Mean	3.96	0.57	85.0

Figures in parentheses show number of children. * Taken from ref. 46. † Taken from ref. 17.

1.57 days and in the ilium 1.35 days. These values refer to the time taken by a cell to move from a crypt to the tip of the villus (see fig. 1); they therefore express the 'turnover' time of the whole intestinal epithelium. Wright, Jennings, Florey and Lium (69) say there can be no doubt that there is a constant and considerable desquamation of epithelial cells into the lumen of the intestine, which is shown by the large number of mitotic figures in the crypts of Lieberkuhn (see fig. 1) and the presence of cells in juice from fistulae even when no food is passing along the gut. It is believed that the only enzymes found in the succus entericus which are truly secreted are amylase and enterokinase and that other enzymes found in the juice are derived from the desquamated epithelial cells.

There is only one study so far as I know that has been made directly on the enzymes of the intestinal mucous membrane, i.e. the work of Heard (23), the results of which are shown diagrammatically in figure 6. The reduction in the amount of this dipeptidase may be crucially important, depending on the extent to which dipeptidase is needed in the final stage of protein digestion (40).

We have produced experimentally in the pig what we

believe to be the analogue of those conditions called in the human marasmus and kwashiorkor by feeding a low protein diet (LP animals) and by feeding the same amounts of this diet to which extra carbohydrate has been added (LP + CH animals) (51) and we find in the pig that zymogen granules in the pancreas are reduced both in the LP and LP + CH animals as compared with the normally fed animal. Also there is some reduction in apparent digestibility in the LP animals and this reduction is more marked in the LP + CH animals. Furthermore, if the LP animals are maintained for some months on the experimental diet more pronounced reduction in apparent digestibility is found, but this quickly returns to normal when a good diet is fed (44). See figure 7; note that the observations on which this figure is based were made at weekly intervals.

In the table some figures are given for apparent digestibility of nitrogen in Gambian infants suffering from protein malnutrition, and showing signs of gastrointestinal disturbance—diarrhoea; also in the table are some figures published many years ago (17) and made on marasmic infants in Glasgow in which the nitrogen intake was about the same as that of the Gambian infants. The Glasgow infants were selected for study because they had no evidences of gastrointestinal upset; the apparent digestibility of this group was normal.

The opening speaker for this Panel, concluded the abstract of his contribution with the statement that "seen from several angles, infant nutrition is still an open chapter." Presumably 'open chapter' means little is known about the subject; we are in substantial agreement with him from the angle of digestion in infancy.

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Nutritional and physiological adaptations in pregnancy

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FOR MANY YEARS, nutrition workers have been concerned about the nutrient requirements of the pregnant woman. It has been thought that pregnancy represents a state of extreme nutritional stress; the pregnant woman must not only ingest nutrients to maintain her own tissues but must also provide additional food to nourish the foetus growing within her uterus. Additional nutrients are needed for the development of the accessory tissues. It has commonly been felt that food intakes which are adequate for the nonpregnant woman may be grossly inadequate during pregnancy. If there is a nutritional inadequacy, then it could be rationalized that deficiency diseases and impairment of obstetrical performance and health of the child would be evident. There have been many studies of the food intake and obstetrical history of pregnant women, and of the paediatric rating of their babies. These have been reviewed elsewhere (19–21, 26, 29, 46, 50, 51) and will not be considered in detail here. Certain changes in the approach to these studies have been apparent. In the early studies, the findings in pregnant women were judged against nonpregnant standards. The fallacy of this approach has been clearly demonstrated by the elucidation, by Macy (30, 32) and by the Vanderbilt Cooperative Study (16), of some of the normal physiological changes in pregnancy.

In the more recent studies of nutrition in pregnancy, employing this new appreciation of the normal physiological changes and more comprehensive study methods, it has become increasingly apparent that obstetrical performance and paediatric rating are not as closely related to nutrient intake as had once been thought. Thus, the early studies of Burke and co-workers (10, 11) relating dietary rating, and particularly protein intake, to the body dimensions and paediatric rating of the infant have not been confirmed by recent studies. Similarly, the report of Ebbs, Tisdall and Scott (18) relating dietary intake to obstetrical performance lacks recent confirmation.

One of the most comprehensive and detailed studies of recent years has been the Vanderbilt Cooperative Study of Maternal and Infant Nutrition, involving somewhat over 2,000 cases, in Nashville, Tennessee. The authors of the many publications arising from this study have tested the relationship of individual nutrients, of high and low planes of nutrition and of combinations of nutrient intakes, to the outcome of pregnancy and the development of pregnancy diseases. The only relationship between nutrient intake and pregnancy diseases they were able to demonstrate was in groups who had dietary intakes, during the third trimester, of less than 1500 calories or less than 50 grams of protein (34). Furthermore, the authors noted that "in both instances the abnormality appears to be responsible for the lowered intake, not vice versa." They could detect no correlation between caloric or protein intake and birth weight or birth length of the infant, nor was there any correlation with complications of delivery or length of gestation. The authors were forced to conclude (35): "In our patients we are unable to indict nutrition as a major causative factor in the development of commonly encountered obstetric and foetal abnormalities. This means either that there does not exist a direct causative relationship between malnutrition and these abnormalities or that our patients were above any such level of nutrition required to precipitate the disease. We are confident that the conditions which occurred among our patients could not have been due primarily to malnutrition. We recognize that with a sufficiently low level of nutrition, nutritional complications may arise from dietary inadequacies. We do not wish to be interpreted as concluding that nutrition is unimportant in pregnancy."

This lack of effect of nutrition, over a wide range of intakes, on obstetrical performance has recently been confirmed by Thomson's studies in Aberdeen (48–50). Dividing his subjects into 2 groups with normal and abnormal clinical histories, and also considering the dietary and obstetrical histories of various social groups, he was unable to relate undernutrition to obstetrical performance. His data suggested that pre-eclampsia was more frequent in persons with a high intake of all nutrients (i.e. in those who overeat). Thomson concluded: "The weight of evidence shows conclusively that the diets of pregnant women can vary widely, in

TABLE 1. *Reported Nutrient Intakes During Pregnancy*

Nutrient	Vanderbilt Cooperative Study (16)	Aberdeen Study, Thomson (48)			Holland Study, Smith (44)			Indian Beggars, Pathak (41)
		Social Class A	Social Class B	Social Class C	Sept. '44	Feb. '45	April '45	
Calories	2020 (640)	2633 (482)	2521 (487)	2354 (513)	1925	731	912	1620
Protein, gm	70 (21)	80.4 (17.2)	78.4 (15.1)	71.8 (15.7)	61	33	39	58
Calcium, gm	1.0 (0.4)	1.19 (0.35)	1.06 (0.32)	0.88 (0.29)	1.08	0.65	0.52	0.32
Iron, mg	13 (4)				16.3	9.6	10.7	12
Vitamin A, IU	5900*	9858 (4334)	8085 (3822)	7545 (3523)	1260	445	766	168
Vitamin D, IU	62*							2
Thiamine, mg	1.4 (0.5)	1.22 (0.27)	1.18 (0.24)	1.16 (0.26)	1.1	1.0	0.6	1.08
Riboflavin, mg	2.3 (0.9)	2.05 (0.53)	1.89 (0.46)	1.74 (0.47)	1.2	0.5	0.5	0.74
Niacin, mg	11 (4)	12.0 (2.8)	11.4 (2.4)	11.6 (2.8)	9.2	3.0	4.1	11
Ascorbic acid, mg	55*	79 (35)	65 (30)	61 (29)	59	34	53	0.2
No. of subjects	2046	101	109	279				20

Mean (Standard Deviation). * Median values.

quantity as well as quality, without clinically obvious impairment of the reproductive process." It was Thomson's feeling that nutrition during childhood, as reflected in body stature of the mother, might be a more important factor than the nutritional intake during pregnancy itself.

A study of preconception diets of women with successful and unsuccessful pregnancies was carried out by Bourquin and Bennum (9). It may be assumed that these diets would reflect the probable type of intake during pregnancy. They were unable to find any striking differences between the groups.

In the majority of the recent studies, including those referred to previously, the average nutrient intakes were not far below recommended allowances (see tables 1 and 2); it should be noted that at the lower ranges of intake in these studies, many of the nutrients were well below some dietary standards. A more severe dietary restriction was seen in some parts of Holland during the war. Smith (44, 45) has reported studies of conditions in Rotterdam and The Hague during the 'starvation period' of the winter and spring of 1944–45. During this period the shortage of food was sufficiently severe to cause amenorrhea in about 50% of the women, and consequently a significant fall in the birth rate later in 1945 and early 1946. It should be noted that the women were relatively well fed prior to the period of food shortage (see table 1). The studies revealed that this severe food restriction during pregnancy led to a slight rise in the prematurity rate, but an accompanying slight decrease in stillbirths and malformations. These changes were so small that they are of doubtful significance; no change in the neonatal death rate was seen. The birth weights of the children were slightly reduced. On the whole, there appeared to be very little interference with the normal process of gestation even under these moderately severe conditions.

A brief report by Pathak (41) described the nutrient intakes of Indian beggars. Included in the group studied were 20 pregnant women. The reported dietary intakes of these women are shown in table 1. Pathak did not comment on the obstetrical performance of these women,

TABLE 2. *Recommended Dietary Intakes in the Second Half of Pregnancy**

Nutrient	Canada (56)	Great Britain (57)	United States (58)	F.A.O. (59, 60)
Calories	2900	2750	2600	2380
Protein, gm	80	102	78	45†
Calcium, gm	1.55	1.5	1.5	
Iron, mg	15	15	15	
Vitamin A, IU	6000	6000	6000	
Vitamin D, IU	400	600	400	
Thiamin, mg	0.9	1.1	1.3	
Riboflavin, mg	1.3	1.6	2.0	
Niacin, mg	9	11	20	
Ascorbic acid, mg	30	40	100	

* Assuming moderate activity. † In terms of reference protein.

but he did state that only one showed any sign of clinical or subclinical nutritional deficiency. Further, he commented that young children were similarly free of symptoms of malnutrition. It would appear that these low intakes did not cause serious disruption of gestation.

That severe or prolonged malnutrition can have an effect on pregnancy is apparent from the studies of conditions during the siege of Leningrad. It has been reported by Antonov (1) that during this siege an acute food shortage persisted for a much longer period than in Holland which was equally or more severe; this food shortage was coupled with poor housing, inadequate heating and in most cases hard manual labour during pregnancy. Antonov reported that during 1942, in the middle of the food shortage, the birth rate had been strikingly reduced, the prematurity rate was increased to 41% of the total live births and 9% of the full term and 31% of the premature babies died in the neonatal period. While it is impossible to separate the effects of malnutrition from those of the many adverse environmental factors, this report suggests that extreme and prolonged food shortage can have a drastic effect on obstetrical performance.

This concept of the effect of extreme malnutrition on gestation is borne out by the many studies in experi-

mental animals. It has long been recognized that severe inanition or prolonged acute deficiency of many nutrients would prevent conception or lead to abortion. A more recent aspect of these studies has been the production of congenital abnormalities. This field has been reviewed by Warkany (52). Skeletal defects have been produced by riboflavin deficiency, soft tissue defects by vitamin A deficiency, and brain and occular defects by other deficiencies. When the antivitamins were employed in these studies, the effects were even more pronounced. However as Warkany points out, great caution must be employed in interpreting these studies in terms of pregnancy in the human. These defects were produced by severe deficiencies, frequently at specific stages of pregnancy. Usually, the deficiency had to be terminated at a specific stage of gestation for the effects to be seen; otherwise abortion resulted. It is likely that genetic factors were also involved since the production of defects varied with species and with strain. Such extreme and specific deficiencies are not likely to be found in a normal human population. Nevertheless, extreme dietary inadequacy may be a factor in congenital malformation. It is unlikely that moderate deficiencies would produce these effects.

The results of recent dietary studies have led to a general disillusionment about the effects of undernutrition on pregnancy. Perhaps this lack of effect should not be as surprising as we might think. In designing dietary standards the customary practice has been to estimate the requirements of the mother on the basis of the nonpregnant woman and to add to this the calculated requirements for the growth of the foetus and accessory tissues. It has now been realized that the pregnant woman is physiologically quite different than she was before pregnancy. Is there any reason to believe that she remains unaltered in the nutritional sense? It seems probable that the lack of effect of nutrition on pregnancy, except under extreme conditions, can be explained on the basis of a change in the metabolism of the nutrients or a nutritional adaptation. There is evidence to support this concept for at least 3 nutrients: protein, iron and calcium.

That the pregnant woman or animal retains additional protein, has been recognized for many years. As early as 1912 Wilson (54) reported that women supplied with generous intakes of protein probably achieved an actual gain in maternal nitrogen stores. The retention of nitrogen was most marked during the last few weeks of gestation. Hunscher *et al.* (27) followed one subject through the last half of pregnancy and lactation. This subject received 18 to 22 gm of nitrogen per day, an exceedingly generous intake, and retained from 2 to 5 g/day. They noted that retention increased with the stage of gestation, independent of intake. The total nitrogen retention during the last 5 months amounted to 446 gm. The loss at parturition was 54.7 gm, the foetus was estimated to contain an additional 58.6 gm and the loss in the lochia during the puerperium was 19.6 gm of nitrogen. Thus, over the total period of pregnancy there was a net gain of 313 gm of nitrogen. A large part of this gain was no doubt due to the high protein intake of this subject; such retentions are not likely on the more moderate intakes seen today. It is known that caloric intake can affect the nitrogen retention of pregnant women (40). Many similar studies have revealed excess nitrogen retentions of high protein intakes. It has been generally assumed that this extra protein represents a storage for some future requirement; there is very little evidence to support this concept. Excess nitrogen retention can be induced in experimental animals by feeding high protein diets. Halac (23) has reported that normal adult rats fed high protein diets retain more nitrogen than do comparable animals on a lower protein intake. In his preliminary studies it was found that when both groups were placed on protein free diets, the high protein-fed rats excreted much more nitrogen than did the other animals, but they survived no longer. The high protein diet, and extra nitrogen retention, afforded no protection against the subsequent effects of protein depletion.

Suitable studies of nitrogen retention of pregnant women on relatively low protein intakes have not been reported. Beaton and co-workers (5) have shown that pregnant rats retain much more nitrogen on unrestricted feeding than do nonpregnant controls, just as do pregnant women. Most of the nitrogen retained can be accounted for by an increased food intake. However, when the food intake (and protein intake) of pregnant rats was restricted to that of nonpregnant animals, the pregnant rats were still capable of storing more protein than were the nonpregnant controls (Beaton, unpublished data). This retention appeared to be accomplished by a decreased excretion of protein, reflecting an increased economy of utilization.

Biochemical studies on pregnant rats have revealed a series of metabolic alterations commencing at midpregnancy, which are believed to favour protein anabolism. It was demonstrated that urea formation was decreased and that several liver enzyme activities and certain blood metabolite levels were altered (4–6, unpublished data). These alterations, shown in table 3, are believed to be under hormonal control. Removal of the foetuses, leaving the placentas in situ did not prevent the alterations; if the placentas were also removed the metabolic alterations were absent at term (4, 15). It was also noted that the pregnant rat exhibited a remarkable resistance to the protein catabolic effects of cortisone (15); a resistance which has also been reported in pregnant women (2, 33). These metabolic alterations were still apparent when the animals were fed diets containing as little as 5% protein (Beaton, unpublished data). This has been reported to be a critical level in pregnant rats (38); intakes below this level can lead to maceration and abortion of the foetuses.

The results of these experiments suggested that there was a hormonal control in the alterations in protein metabolism in pregnancy. It was found that in nonpregnant rats, administration of growth hormone,

TABLE 3. *A Comparison of Effects of Pregnancy and of Growth Hormone Administration in the Rat*

	Pregnancy (Last Half)	After Growth Hormone
Nitrogen retention	increased	increased
Liver alanine-glutamic transaminase activity	decreased	decreased
Liver aspartic-glutamic transaminase activity	unchanged	unchanged
Liver catalase activity	decreased	decreased
Liver D-amino acid oxidase activity	decreased	decreased
Liver urea production	decreased	decreased
Liver ketone body formation	increased	increased
Blood ketone bodies	increased	increased
Blood urea	unchanged*	decreased (slightly)
Blood amino nitrogen	decreased	increased

* Pregnant rats exhibit a decreased rate of removal of urea from the blood (4).

TABLE 4. *Haemoglobin and Packed Cell Volumes During Pregnancy*

Time of Gestation	(1) Haemoglobin, gm/100 ml	(2) Packed Cell Volume, %	Ratio of (1) to (2)
	A. Human (16)		
5–9 weeks	13.1	39.2	.334
10–13 weeks	13.0	38.2	.340
14–19 weeks	12.0	35.8	.336
20–26 weeks	11.9	35.5	.346
27–32 weeks	11.4	34.8	.328
33 weeks and over	11.6	35.1	.330
	B. Rat (5)		
5 days	12.0	51.0	.235
8 days	11.7	50.5	.232
12 days	11.2	45.7	.245
14 days	11.2	44.4	.255
16 days	11.6	47.3	.245
18 days	10.9	42.0	.259
20 days	10.3	41.0	.252
22 days	10.5	39.5	.266

especially in the presence of estrogen and progesterone could produce many of the biochemical alterations seen in the pregnant rat (6, unpublished data). A comparison of some of the effects of pregnancy and of growth hormone is seen in table 3. The changes produced by growth hormone, even in the presence of the sex hormones, have not been as great as those seen in the pregnant rat. The discrepancy in the effect on blood amino nitrogen may be explained by the presence of an active transport mechanism across the placenta in the pregnant rat; this fall is abolished by removal of the foetus (4). These studies suggest that the anterior pituitary growth hormone may be involved in the series of alterations which decrease the catabolism of protein during pregnancy. Further support for this hypothesis has come from the detection by Contopoulos and Simpson (13), of an increased amount of a growth-promoting substance in the blood of pregnant rats. These workers felt that the maternal and foetal pituitaries and the placenta were all involved in the production of this growth promoting substance.

Whatever the mechanism may be, it is certain that in the pregnant rat there are metabolic alterations which promote the retention of protein. As protein intake increases, more nitrogen is retained (42), but it is also apparent that these metabolic changes function and are probably of greater significance on low protein intakes during pregnancy. It has been reported that gestation can be continued on a protein-free diet after mid-pregnancy (43). There is little evidence with regard to the metabolic alterations in the human, since suitable enzymatic studies have not been possible. McGanity *et al.* (36), and others have reported that blood urea is lowered in pregnant women; after delivery there is a prompt return to nonpregnant levels. This lowering was not due to increased urea clearance or to haemodilution. Their data suggested that urea formation was decreased in the pregnant woman, as evidenced by a smaller increase in the blood urea of pregnant than of non-pregnant women following the administration of alanine. A similar response to injected amino acids has been reported in the pregnant rat (4). Lowered blood amino nitrogen levels have also been observed in pregnant women (7), just as they have in the pregnant rat. It is likely that metabolic alterations occur in the pregnant woman similar to those seen in the pregnant rat. Further studies of the metabolism of women maintained on relatively low protein diets will be needed to fully determine the importance of this form of adaptation.

Studies of iron metabolism in the human and in the experimental animal have revealed definite physiological and nutritional adaptation during gestation. This subject has recently been reviewed by Hytten and Duncan (28). It is now recognized that the physiological increase in blood volume accounts for the prominent fall in haemoglobin level during gestation. The fall in haemoglobin is directly proportional, under normal conditions, to the fall in packed cell volume, as is shown in table 4. The haemoglobin content of the red cell shows little or no change, and since the total number of circulating red cells increases, the actual total amount of haemoglobin also increases during pregnancy. This normal physiological phenomenon of pregnancy in no way reflects a deficiency of dietary iron (28).

In considering the iron requirements of a pregnant woman, other physiological changes must be remembered. The normal nonpregnant woman loses approximately 25 mg of iron in the menstrual flow (37). During the period of gestation the cessation of menstruation represents an iron economy amounting to 200 to 300 mg of iron. In addition to this conservation of iron, several workers have demonstrated, using radioactive iron, that iron absorption is increased during pregnancy (3, 22). This increase may be 3- to 4-fold during the last trimester of pregnancy. It is recognized that iron absorption is affected by the body's need for iron. It has been suggested that iron absorption is dependent on the relative saturation of the iron-binding protein of plasma,

transferrin (12). Preliminary studies suggest that this protein increases in the serum of women as gestation progresses (Beaton, unpublished data); this may be an explanation of the increased efficiency of absorption in pregnancy. It is probable, therefore, that significantly increased amounts of iron can be absorbed during pregnancy without any increase in iron intake.

Unless there is a severe loss of blood, true iron-deficiency anemia is fairly uncommon in pregnancy. In the Vanderbilt study (34), anemia was classified as a haemoglobin level below 10 gm/100 ml. Such levels were encountered in only 10.5% of the pregnant women who had not suffered blood loss. Further examination of the bloods of these women suggested their low level of haemoglobin could be attributed to a greater than normal increase in blood volume in the majority of cases. Only 37 women (total survey 2,046) had an anemia which could be attributed to iron deficiency on the basis of blood indices. Woodruff and Bridgforth (55) selected women from the Vanderbilt study who had haemoglobin levels above 12.7 gm or below 10.7 gm and observed the haemoglobin levels of the children. They could find no evidence of a consistent difference in haemoglobin levels of the infants at birth, or at any time during the first year of life. They concluded that in the absence of significant iron deficiency, there is no correlation between maternal haemoglobin level and the development of hypochromic anemia in infancy. Thus, although it is known that supplemental iron administration will raise the haemoglobin levels in pregnant women (8, 53), just as it will in nonpregnant adults, there is no evidence that this is of any value to either the mother or the infant in the majority of cases. The treatment of acute iron deficiency anemia (average haemoglobin = 5.3 gm) with iron can have a beneficial effect as was shown by Strauss (47).

It would appear, then that the iron requirement in pregnancy is minimized by at least 2 factors: *a*) the conservation of iron brought about by the cessation of menstruation, and *b*) the increased efficiency of absorption of dietary iron.

Calcium metabolism is also subject to adaptive changes in the pregnant woman. Several workers have pointed out the mechanism of nutrition adaptation to low intakes of calcium in children and normal adults. Hegsted (24) indicated that the calcium requirement of an adult was proportional to the habitual dietary intake. Men who were accustomed to low intakes over a prolonged period of time were able to maintain calcium balances on as little as 100 to 300 mg of dietary calcium per day. In Ceylon, it was shown that children can adapt to intakes of calcium of 200 mg/day by increasing the absorption to as much as 89% of ingested calcium and markedly decreasing the excretion of calcium (39). Comprehensive studies of calcium metabolism in pregnant women on low intakes have not been carried out, but increased calcium absorption in late pregnancy has been reported (14, 17, 31). In view of the low calcium intakes observed in many of the surveys of nutrition in pregnancy, it seems apparent that such an adaptive mechanism must function.

The available evidence indicates that adaptive changes take place in the pregnant woman to minimize the requirements for protein, iron and calcium. It is not known whether any adaptive mechanisms exist for the vitamins. It has been reported, without confirmation, that vitamin B_{12} absorption is increased in both the pregnant woman and the pregnant rat (25). Increased absorption or altered metabolism of other vitamins may occur in pregnancy.

In reviewing the physiological changes in pregnancy, Leitch (29) made the following statement: "To establish the idea that an organism, here in particular a woman when she becomes pregnant, can set up an entirely new equilibrium in the internal milieu and that over, say, 1000 women there is almost an incredible range in the extent of the changes that occur, let me summarize briefly what happens. She, the pregnant woman, may put on no weight at all, or 45 lb. or more, and, doing so, may produce a baby at term that weighs anything from 5 to 10 lb. without much relation to her own weight gain. She will increase the volume of her plasma by anything between 14 and 121% and her total extracellular water by nearly 3 times as much. She will manufacture new red cells sufficient to raise total circulating haemoglobin by about 15% with less variation than in plasma volume, and, in order that oxygen may be unloaded at higher tensions and carbon dioxide more readily picked up, she will overbreathe, raise the hydrogen ion concentration of her blood and shift the oxygen-dissociation curve of her blood to the right. All of that adds up to a highly pathological condition by non-pregnant standards, but while so handicapped (by nonpregnant standards) the pregnant woman will improve her digestion and absorption and go about her ordinary business. And, having built up this new 'way of life' to a climax at 36 weeks, she will undo most of it during the next 6 weeks. Clearly, we require criteria of normality for pregnancy that will be relevant to the events of pregnancy."

In the present review, an attempt has been made to describe some of the metabolic alterations of pregnancy. The implications of these alterations or nutritional adaptations must be clear. Not only can we not interpret the physiology of the pregnant woman in terms of that of the nonpregnant woman, we can not interpret the nutritional requirements of the pregnant woman in terms of those of the nonpregnant woman. It is apparent from both animal and human studies that there are minimal nutrient requirements; if these are not met, harm to the mother or the foetus will result. Above this minimal level, the pregnant woman can adapt to a wide range of nutrient intakes. Not enough is known, yet, to define the limits of this range of nutrient intakes. It is apparent that we are seriously in error if we neglect these adaptations in considering the nutritional status and requirements of the pregnant woman. The simple

process of adding maternal (nonpregnant) and foetal (and accessory tissue) requirements gives an erroneously high estimate of the nutritional requirements of the pregnant woman.

It is this failure to recognize the physiological and nutritional adaptations of pregnancy that has led to the disillusionment in studies of the effects of dietary intake on the outcome of pregnancy. Fourteen years ago, Garry and Wood (21) stated: "But it is quite certain that reproduction is not in itself a pathological process and that, if pregnancy and lactation seem to require inevitable routine mass therapeutic measures, then we may be sure that something is wrong with modern living or in the interpretation of our information." Today it is evident that it is our information that has been misinterpreted.

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Nitrogenous constituents of urine in kwashiorkor[1]

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In BALANCE STUDIES on children recovering from kwashiorkor that were intended to provide comparisons of the effectiveness of a milk diet (consisting of calcium caseinate, dried skim milk, sucrose and cottonseed oil) and a diet of biscuit meal (made from peanuts, wheat and corn flour, dried skim milk, sucrose and cottonseed oil), an attempt was made to correlate the utilization of the dietary N with the output of urea N. The attempt failed, but it was noticed that in several children, including some who had received only short periods of treatment, the percentage of the total urinary N that appeared in the form of urea was considerably lower than the normal 87 to 93.

It seemed advisable to investigate the urea output of the children when they were admitted and after they had received a course of apparently successful treatment. It was obvious that the urea output had to be related to the total output of N in the urine, and to minimise the effects of fluctuations in urea concentration, collections of urine were made for 12-hour periods on the day of admission and again when clinical and biochemical signs showed that treatment was successful, 2 to 4 weeks later.

Nothing was known for certain about the diet of the children immediately before admission, although nearly all the mothers complained that the appetite had been poor, and from their accounts it seemed that very little protein-rich food could have been eaten. Standardisation of the diet during the first collection period was thought to be necessary, and it was achieved by giving nothing but glucose water, a procedure that was known from much experience to be innocuous and in no way to endanger rapid recovery. Some children, however, were given one of the diets mentioned previously during the first period.

It is our practice to record a clinical grading of children at their first examination into categories of severe, moderate and slight kwashiorkor, and we have shown (20) that the gradings usually correspond to different levels of total serum protein. For the present investigation, an arbitrary distinction was preferred: cases were classed as 'severe' if the total serum protein was less than 4.5 g/100 ml, and 'less severe' if it was higher (from 4.5 to 5.6 g/100 ml). The resulting division corresponded reasonably well with the clinical grading.

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RESULTS

Total N excretion. Fourteen children were given nothing but glucose water during the first collecting period, and their average N output was 549 mg for 12 hours. There was, however, a large difference between the 8 'severe' and the 6 'less severe': one averaged 726 mg, and the other 311 mg.

Eight children were given 1 of the nitrogenous diets during the period. The average N output was 632 mg: 4 'severe' averaged 715 mg and 3 of the 'less severe' averaged 333 mg, but the output of the 4th was 1200 mg. There was, therefore, a small increase in the N output that was associated with the N intake, but the degree of severity of the child's condition was apparently much more important than the kind of food given in determining the extent of the output.

As the intake did not affect appreciably the results obtained, the 12 'severe' and 10 'less severe' cases have been summarised without reference to diet in table 1. The table includes data obtained after treatment. Two children (those numbered 1 and 11 in table 2) had the biscuit meal diet and all the others had the milk diet, both diets providing about 5 g protein per kg body weight. The effect of treatment was to increase the total N output in the urine to about 1235 mg in 12 hours, an amount that was probably not highly abnormal.

Urea-N. The table shows that in the first period, the amount of N appearing as urea was less than 60% of the total, with little difference between the 'severe' and 'less severe' cases. After treatment, the percentage rose to 90.

α-Amino N. The range of excretion of amino-N was large, and the differences shown in the table are not statistically significant. Other workers, who have investigated chiefly the free amino-N of the urine, have reported raised values: our figures, that are for the total of free and combined (peptide) amino-N, do not suggest an increase.

In a few children in whom combined and free amino-N were estimated separately, the ratio of combined to free fell during treatment. The cause was a fall in the combined N, rather than a rise in the free.

Ammonia-N. The ammonia-N excreted by the 'severe'

TABLE 1. *Average N Excretion by Children Before and After Treatment for Kwashiorkor (mg N/12 Hours)*

Condition of Children	Untreated 'Severe'	Untreated 'Less Severe'	Treated
Number	12	10	10
Total N	722	404	1235
Urea-N	408	242	1117
α-Amino-N	33	24	27
Ammonia-N	64	33	41
Creatine-N	18	5	9
Creatinine-N	22	15	10
Uric acid-N	9	8	11
Total of 6 constituents	544	317	1215
N not accounted for	178	87	20

TABLE 2. *Excretion of Uric Acid-N and Other Purine-N Before and After Treatment (mg/12 Hours)*

Child No.	Uric Acid	Before Treatment					After Treatment		
		A	B	C	D	Total of A, B, C & D	Uric acid	A, B, C	D
Severe									
1	8	5	11	23	13	52	7		2
2	13		8	27	9	44	13		
3	8	13	29			42	8		
4	9	10	19		14	43	12		
5	9	40	16		18	74	13	not de-	1
7	11	3	6		16	25	10	tected	3
11	10		1		2	3	15		2
12	10		3		8	11	13		
Less Severe									
13	9		17		9	26	12		
14	8	2	4	17	12	35	9		

cases was nearly twice that excreted by the 'less severe'. The averages are misleading: 1 child in the 'less severe' group excreted 105 mg in the first 12 hours, and if that result is omitted, the average for the group falls to 25 mg. Our results support the suggestion made by Smith (3) that the kidney of the malnourished child has a reduced ability to produce acid.

Creatine and creatinine-N. The amounts of creatine and creatinine excreted by the 'severe' group were considerably greater than those excreted by the 'less severe'. There was very little overlap between the 2 sets of results for creatine, but some evidence was obtained for the presence, in the 'severe' group, of coloured substances, of unknown nature, that may have contributed towards spuriously high values. The possibility is being examined further but it is worth noting that only the urine of the 'severe' cases appeared to be affected.

In the short period of treatment that we observed, the values for creatine and creatinine usually fell considerably (see table 1). Over a much longer period of treatment, Standard *et al.* (4) found a large rise.

Uric acid-N. Uric acid was estimated as it was the only other common urinary constituent that might have contributed largely to the total urinary N. As table 2 indicates, the excretion rose slightly in about half the children who were studied at the beginning and end of treatment. The significance of this observation must for the moment, however, be regarded as doubtful.

N not accounted for. Table 1 shows that in the 'severe' cases 178 mg, and in the 'less severe' cases 87 mg, of the total N on admission was not accounted for by the 6 substances estimated: these amounts represented a little more than 20% of the total. After treatment, only the very small amount of 20 mg, which was well within the combined experimental errors of the estimations, remained. Obviously, a large change had occurred.

Nature of the unaccounted N. A systematic exploration of the urine for nitrogenous substances known to be excreted as the result of faults of metabolism was begun, but was deviated towards purine metabolism because it was recalled that Waterlow (6) had reported a reduced RNA/DNA ratio in acute malnutrition. Ultra-violet spectrophotometry of purines gives characteristic curves, and purines can be separated by paper chromatography, and with these techniques it has been possible to demonstrate a series of substances in the urine of children suffering from kwashiorkor, that are probably purines. Details of 4 of the substances that appear most consistently and in the largest amounts are given in table 2. The nomenclature A, B, C and D is a temporary expedient. It corresponds to increasing R*f* values, but does not denote several intermediate substances that have been detected, usually in very small amounts. The values given for the 4 substances are only approximate; they have been derived from the height of the spectral curves, by the standard method that uses uric acid as a reference material, on the assumption that the molar extinction coefficient of the unknown substances is the same as that of uric acid. The true values might be half as great again as those shown in the table; it is unlikely that they could be lower.

The lengthy and exacting process of identification of the substances has begun. It cannot be completed for many months, but some information can already be given:

a) Substance A. At pH 1, this substance has its maximum absorption at 268 mμ, and at pH 12, its maximum absorption at 285 mμ. Hydrolysis with HCl has no effect: a positive reaction is obtained with mercuric nitrate and ammonium sulphide (Vischer and Chargaff, (5)) and a blue colour is produced by the silver nitrate-bromophenol blue reagent (7). It is probably a simple purine, and a derivative of xanthine. Its absorption spectrum resembles that of 2:8 dimethylxanthine.

b) Substances B and C. The maximum absorptions of substance B in acid and alkali are at 273 and 260 mμ, respectively, but those of substance C are at 277 mμ in both acid and alkali. On hydrolysis with HCl both substances disappear, and there is an increase in uric acid. The substances are probably purines closely related to uric acid.

c) Substance D. In alkali, this substance has its maximum absorption at 270 mμ, and in acid, at 260 mμ. On hydrolysis with HCl it disappears, and a new compound appears that has its maximum absorptions at 270 and

280 mμ in acid and alkali, respectively. It is probable that substance D is a xanthine derivative.

d) Other substances. Between substances C and D in the chromatograms of several children a substance has been found that has maximum absorptions at 253 mμ in acid and 290 mμ in alkali. It disappears on hydrolysis with HCl, and a new substance appears, with the same maximum absorption in alkali but a maximum of 270 mμ in acid. The substance may be related in some way to a dietary intake of peanuts: it has been found chiefly in the urine of children who were given a diet containing peanuts or who may have had peanuts before admission. Its nature is unknown.

A substance that moves in the chromatogram with substance D has been detected several times, but only once in a large amount. It is chiefly of interest because on hydrolysis it gives rise to a new substance that resembles a derivative of hypoxanthine in its properties.

Effect of Treatment

With successful treatment the abnormal purine-like substances disappear almost entirely from the urine. As shown in table 2, substance D is usually the only one that persists in measurable amounts.

DISCUSSION

Some speculations on the meaning of the results given may be justified.

The large losses of N by the child who is acutely ill and already depleted of protein—increasing with the severity of the condition—must produce an intolerable strain that must quickly become insupportable. It may be possible to gauge the effectiveness of therapeutic diets by the speed at which they halt the losses.

In theory, much could be explained on the basis of the renewal of enzyme activity brought about by the availability of new protein derived from the diet, either for enzyme synthesis or for substrate formation. If the urinary α-amino N is an accurate index of the ability to deaminate amino acids, there may be a slight inability to complete the breakdown of peptides, but little wrong with deamination itself; but the excretion of large amounts of ammonia, although it may be related to a failure of acidification by the kidney, may show that the xanthine-citrulline-arginine cycle is defective. Ammonia is being produced that cannot be carried to urea, and so the urinary ammonia is high and the urea low. (It is, incidentally, well known that in severe kwashiorkor the blood urea is usually very low indeed.)

The increase in excretion of creatine and creatinine suggests the breakdown of muscle cells, which are the chief source of these substances; and the discovery of the purines may indicate that intracellular nucleic acids are being lost, perhaps because of a failure to use material that is constantly being produced by the vital catabolism of the nucleic acids themselves, perhaps because of a failure to maintain the integrity of the cell wall. An alternative possibility, that the abnormal excretion may represent a stay in synthesis of the nucleic acids, will, it is hoped, prove amenable to a dynamic study.

The excretion of the substances believed to be abnormal purines is extremely interesting on several counts. It occurs more often, and to a greater degree, in severe cases of kwashiorkor than in slight ones, and it is much greater in amount (as is shown in table 2) than the excretion of the common purine, uric acid. A deficiency of xanthine oxidase has been demonstrated in the livers of children with kwashiorkor by Burch *et al.* (1); it might be responsible for the appearance of the abnormal substances, in place of the normal end-product.

There is a tendency for the output of the abnormal purines to be greatest in those cases in which the unaccounted N is also highest, but a comparison of tables 1 and 2 shows that the purines do not usually account for more than about a quarter of that N. Amines and other substances are being considered for the gap, and it is perhaps best to regard this report as no more than the beginning of another attempt to discover the essential lesion of kwashiorkor. It will be surprising, however, if eventually the severity and the prognosis of kwashiorkor are found not to be closely related to the purine excretion.

The members of the staff of the Unit who have carried out the work are Dr. Roger Whitehead and Miss Celia Matthew.

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Panel Summary—Maternal and Infant Feeding

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IT IS QUITE APPARENT that our presentation today has emphasized the fact that we have as yet but little knowledge of the subject of nutrition in maternal and infant feeding, so that Dr. Dean's remark that we need further scientific experiment, I think, runs like a thread through the whole of our discussion.

Another point that has been brought out is that, while it is reasonable to envisage the nutrition of the mother and child as a continuum, further information is needed. In this regard it is clear that the panel believes in the old saying that infant feeding begins *in utero*, so much so that in parts of China the child was regarded as being one year old at birth. However, we do need to prove the links in this continuum.

If I may, I should like to emphasize certain points which seem to me to be of significance and require particular further study and research in the future.

Nutrition in pregnancy and lactation received considerable emphasis. What, for example, is the effect of nutrition (as Professor Platt has queried) on early breast development? What is the effect of nutrition on the fetus? What is the relation of a low birth weight tropical neonate to nutrition and to other environmental and genetic factors. How does nutrition affect lactation? How does nutrition affect the later life and health of the lactating mother, especially if the process is repeated frequently? What is the relation, if any, between nutrition and the toxemias of pregnancy?

Dr. Beaton's paper stressed very much that we do not know the nutritional needs of proximate principles, vitamins and so forth, during pregnancy. What are the extents of nutritional adaptation during this period? What are the limits to which the woman can adapt to adverse nutritional intakes?

These are some of the questions which we have to answer. Others will include: how do we measure malnutrition during pregnancy? Should one use simple parameters of disease? Can one use the weight or selected biochemical tests?

Further work is required concerning human milk. In general, it seems to be the feeling, although difficult to prove, that, teleologically, human milk is best adapted to the human infant. It seems highly likely that there may be nutritional differences between it and cow's milk, and, indeed, anti-infective factors, as implied by Dr. György. The nutritional composition of human milk requires further study, as suggested by the pioneer work carried on by Dr. Gopalan, Professor Platt, and others. In other words, we need to know more about human milk not only from the point-of-view of total protein, but also with regard to other newer concepts, for example, curd-whey ratio, which have been brought to light by our panel discussion.

It has been stressed by the speakers that it is easier to carry out experiments on animals than on women, but nevertheless these studies will have to be planned.

The relation between underfeeding and lactation received considerable emphasis, and rightly so, as it lies very much at the core of so many problems of tropical pediatrics. What is the relation between the diet in lactation and the quantity and the quality of the milk, especially in regard to the newer concepts already referred to? This certainly requires further investigation.

The ability of unsophisticated under-fed mothers to lactate has been stressed, but we also have mentioned the need to investigate the reasons for the failure of lactation which is tending to erode and to creep into the wealthier groups in the tropical regions, and also, regrettably, in some areas is commencing to affect poorer mothers as well.

What is the effect of lactation on the mother? How does it affect her weight and her general nutrition, especially if, as Dr. Gopalan noted, this is a continuous process throughout her life.

With regard to cow's milk, it seems to be generally accepted that, in the technically developed western world, clinically satisfactory results can be obtained with feeding with 'protein-high' cow's milk. Accelerated growth can be achieved, which can be a cultural goal in a part of the world which is highly competitive. Dr. György has posed the question: what is the late effect of this overload, if it is, indeed, an overload? Does it have any relation to the development, as Dr. Platt has suggested or implied, to the prevalence of peptic ulcer later in life? Does it have any relation to some of the metabolic disorders of the central nervous system which appear to be commoner in non-breast feeding areas of the worl?

Digestion has been mentioned briefly, especially some very interesting work on the digestion of milk—the fact that whey leaves the stomach first. Various other factors have been brought out. All of these, I think, need wider publicity and also may be found to have practical applications.

Supplementary feeds were discussed briefly. The time to introduce these would seem to vary in different parts of the world. There seems to be a feeling that the over-

early emphasis on the introduction of mixed feeds in some parts of the world, especially in the United States and Western Europe, may be both unnecessary and possibly have psychological ill effects. In tropical countries, one has, it seems, to base one's ideas of infant feeding on general principles which are universal, but to adapt these to the local circumstances. As a generalization, in the poorer majority in the tropics, probably the time to introduce supplementary feeds is in the beginning of the second semester of the first year of life.

Protein requirements in infancy were touched upon and it was pointed out that further work is needed even in this important and central area. There seemed to be some difference of opinion as to the requirement, but Dr. György suggested a range of 2–3 g/kg of body weight per day.

These, then, are the points which have struck me as being important. I would, however, if I may, like to finish by quoting something which seems to come very much to the core of our discussion in relation to human milk and lactation, and which was written as long ago as 1873 by Anthony Trollope, the novelist. He said and I would like to leave this thought with you, "How is it that women with little food can suckle their babes, while those who are coshered up with fowl and port cannot, we will leave to the physicians to decide."

PANEL V

Effects of Processing and Additives on Foods

Chairman: C. A. Elvehjem, USA
Moderator: W. J. Darby, USA

Nutritional effects on milk of chemical additives and processing

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BEFORE I CONSIDER the effects on it of additives and of different forms of processing it will be helpful if I state briefly the salient facts about the composition of milk and its part in human nutrition.

Various aspects of the nutritive value of milk, including the effects of processing, have been studied extensively over many years in my laboratory and I have listed this work and that of others in a series of reviews covering the years 1934 to 1954 and in two later ones (36–45). Some of the recent work at Shinfield on the effects of processing is described in (8, 10, 18, 24).

Milk is a labile complex mixture consisting of an emulsion of fat and a colloidal dispersion of proteins together with the milk sugar, lactose, in true solution. These major constituents are accompanied by various minerals, notably calcium and phosphorus, vitamins, enzymes and various organic compounds such as citric acid, some of them nitrogenous in nature, together with dissolved gases, carbon dioxide, nitrogen and oxygen. As that of any other vegetable or animal product, the composition of milk varies markedly. It is affected by the breed of the cow, the physiological variability of the individual, its nutrition, stage of lactation and age, the season of the year, amount of milk produced and so on. Values quoted for 'typical' composition can have only a representative statistical meaning, and it is in this sense that the values given in table 2 for Friesian-type milk can be taken. It may be useful to gauge the value of milk in the human diet by an assessment of the contribution a reasonable quantity of it makes to the nutrient requirements as defined by a recognized authority. Table 1 shows in this way the contribution of 0.5 litre of milk daily to the requirements of a child aged 2–6 years and of a man doing medium work, as defined by the British Medical Association (6). It will be seen that the importance of milk lies largely in the protein, calcium, riboflavin, vitamin A and thiamine it contains. In fact, surveys of national diets show that, in those communities where milk and milk products are a customary important part of the diet, they supply some 15–30% of the total protein, often over 50% of the total calcium intake, up to 20% of the vitamin A and some 40–50% of the total intake of riboflavin.

Though, potentially, the vitamin C contribution of milk is also large, the marked destructive effects of handling, discussed later in this article, make reliance on this source unsafe. The values quoted in tables 1 and 2 do not do full justice to the protein contribution of the milk or to its value as a source of nicotinic acid. The milk proteins are of high biological value, i.e. their amino-acid pattern is such that they can be efficiently used for the building and replacing of body tissues. Moreover, they exhibit marked complementary relationships with the proteins of cereals and starchy staples. In contradistinction to that in cereals (5) the nicotinic acid in milk is all in the free, available form; moreover, milk proteins contain enough tryptophan to contribute by conversion more of the vitamin.

The main recognized shortcomings of milk are that it is deficient in iron and low in vitamin D.

Milk is by nature and composition a most perishable product and, save in small pastoral communities, cannot serve as foodstuff without some form of processing. The purpose of the processing is the destruction of noxious microorganisms whether present originally or by later contamination and the preservation of milk for a reasonable time in a safe and palatable state.

Most of the methods of processing, and there are many, involve the application of heat which in some measure affects certain important labile nutrients in milk. The concomitant changes in the nutritive value, appearance and taste of milk are as unavoidable as those in the making of other components of the varied dietary of civilized man and equally acceptable in modern dietetics and food technology.

As with other foodstuffs, chemical additives have also their part to play in the preparation of milk for human consumption. Some are added to milk for purposes broadly similar to those of heat processing, or ancillary to it, and others are intended to increase the nutrient value of milk. Apart from such intentional additions certain potent chemicals are increasingly finding their way into milk as a result of new developments in agriculture, warfare and technology and some consideration must be given to them. Finally, under this head might come also adventitious metals and

TABLE 1. *Approximate Percentage Contribution of 0.5 Litre Fresh, Raw Summer Milk to the Recommended Dietary Allowance for a Child and for a Man**

Nutrient	Child, 2–6 Years	Man, Medium Work
Calories (half from fat)	22	11
Protein	31	20
Iron	2	1
Calcium	60	76
Vitamin A (in fat)	22	14
Vitamin D (in fat)	3	
Thiamine	35	18
Nicotinic acid	7	4
Riboflavin	87	44
Vitamin C	70	52

* Recommended dietary allowance for a child aged 2–6 years and for a man doing medium work as defined by the British Medical Association (6).

other substances reaching milk in the processing plant and machinery.

CHEMICAL ADDITIVES

General Remarks

Several of these additives, particularly the unintentional ones, constitute potential or established food hazards; moreover only a very few of them are essential to the utilization of milk as food. In most countries, therefore, additives are covered by a strict legislation and in many countries regulations prohibit the addition of any substances to milk whatever their nature or purpose.

Many countries, and such official bodies as the FAO, look with disfavour on the use of chemicals for purposes well served by heat treatment. Thus a meeting of experts on the use of hydrogen peroxide and other preservatives in milk, convened by FAO in Interlaken, Switzerland, in September 1957, was of the opinion ". . .that, in general, the use of any preservative in milk is undesirable —in fact that the addition of any preservatives to milk can only be regarded as being of the nature of a necessary evil. It is a method to be tolerated only in exceptional circumstances, and in warm or technically less well-developed countries where rapid transport of producers' milk to a processing centre is not possible, or where effective cooling of the milk cannot be carried out and where, if a preservative were not used, serious loss of human foodstuff would result" (12).

Intentional Additives

For preservation. HYDROGEN PEROXIDE. In tropical and subtropical countries and in some warm European countries, hydrogen peroxide has been successfully used, with official blessing or without, for preservation of liquid milk. The procedure has been reviewed and studied in detail by Lück (47–49).

Whatever the bacteriological and hygienic value of the treatment, its nutritive effects are remarkably small, particularly if the excess of hydrogen peroxide is removed by catalase as soon as the requisite length of exposure is reached. Of the vitamins measured, β-carotene, vitamin A, tocopherol, thiamine, riboflavin and pyridoxine were not affected but vitamin C was almost completely destroyed. The destruction of the proteins as measured by reduction in sulphydryl content was, if anything, less than in holder pasteurization. Other experiments, in which milk was treated with hydrogen peroxide for up to an hour and then pasteurized, showed no loss of thiamine, riboflavin, nicotinic acid or vitamin B_{12} as compared with that due to heat treatment alone (56).

QUATERNARY AMMONIUM COMPOUNDS. Attempts have been made to use these materials for preservation of liquid milk, with little success. Their number is so great and their effect so varied that it is difficult to generalize. Clegg and Sarjeant (9) found that concentrations greater than 50 ppm were necessary to give even small improvements in keeping quality and, since bitter taints frequently developed at much lower concentrations, there seems little possibility of the successful application of the compounds.

SODIUM HYDROXIDE, SODIUM BICARBONATE AND OTHER NEUTRALIZERS. These chemicals are used to reduce acidity arising out of bacterial action so that the milk can be further treated by heat without clotting. There is little or no information about the effects they may have on their own.

For enrichment. GENERAL REMARKS. It is agreed that certain shortcomings of national diets or of diets of selected groups of people are most conveniently made good by addition of the deficient nutrients to selected foods, chosen because they are widely consumed in significant quantity, as, for example, by the addition of calcium and vitamins to bread or the iodization of salt.

VITAMIN D. With official approval and support in several countries, milk and its products have for some years been used as convenient carriers of vitamin D, particularly for infants and young children.

The enrichment is easily achieved by dispersion in milk of suitable solutions of vitamin D_2 or vitamin D_3, can be carried out at a suitable stage of milk processing and, apart from adding vitamin D, does not affect the nutritive or other qualities of the finished product. Such fortification is extensively practised in the United States of America where a large part of the bottled milk and almost all evaporated milk sold contains added vitamin D and is used in many other countries including Sweden, the United Kingdom, Germany, Czechoslovakia, Switzerland and the U.S.S.R. Naturally, such enriched milk must be used intelligently, particularly when it is intended as the almost sole food for infants. In the United Kingdom lately there has been some increase in infant hypercalcaemia and the health authorities have recommended (55) that the vitamin D content of enriched National Dried Milk distributed under welfare schemes be lowered from 1000 to 350 IU/100 g. Infants and young children have access under these schemes, apart from that in the National Dried Milk, to vitamin

TABLE 2. *Composition of Different Forms of Cow's Milk, Liquid, Concentrated and Dried, and Losses of Nutrients in Manufacture*

Form	Water, %	Protein (N × 6.38), %	Fat, %	Carbohydrate, %	Calcium, %	Vitamin A Activity		Vitamin D		Thiamine		Riboflavin		Pantothenic Acid		Nicotinic Acid		Vitamin B_6		Biotin		Vitamin B_{12}		Vitamin C*	
						Amount	Loss %	Amount	Loss %	Amount	Loss %	Amount	Loss %	Amount	Loss %	Amount	Loss %	Amount	Loss %	Amount	Loss %	Amount	Loss %	Amount	Loss %
						IU/100 g		IU/100 g		mg/100 g		mg/100 g		mg/100 g		mg/100 g		mg/100 g		mg/100 g		mg/100 g		mg/100 g	
Whole milk																									
Raw	87.6	3.3	3.6	4.7	0.12	150		2		45		150		350		100		35		1.5		0.30		2.0	
Pasteurized: holder process	87.6	3.3	3.6	4.7	0.12	150	None	2	None	40	10	150	None	350	?	100	None	35	None	1.5	None	0.27	10?	1.6	20
H.T.S.T.	87.6	3.3	3.6	4.7	0.12	150	None	2	None	42	<10	150	None	350	?	100	None	35	None	1.5	None	0.30	<10	1.8	10
Sterilized: in-bottle process	87.6	3.3	3.6	4.7	0.12	150	None	2	None	30	35	150	None	350	?	100	None	18	50	1.5	None	Trace	>90	1.0	50
U.H.T. alone	87.6	3.3	3.6	4.7	0.12	150	None	2	None	42	<10	150	None	350	?	100	None	35	None	1.5	None	0.24	20	1.8	10
U.H.T., then in-bottle process	87.6	3.3	3.6	4.7	0.12	150	None	2	None	25	45	150	None	350	?	100	?	18	50	1.5	None	Trace	>90	<1.0	>50
Evaporated †(a)	68.5	8.4	9.2	12.0	0.30	375	None	5	None	67	40	375	None	875	?	250	?	35	60	3.4	10	<0.10	90	2.0	60
(b)	73.0	7.0	8.0	10.0	0.26	315	None	4	None	57	40	315	None	735	?	210	?	29	60	2.8	10	<0.10	90	1.7	60
Sweetened condensed: (a)	25.0	8.4	9.2	55.4	0.30	375	None	5	None	103	10	375	None	875	?	250	None	88	None?	3.4	10	0.53	90	4.3	15
(b)	29.0	7.3	8.0	53.9	0.27	330	None	4	None	90	10	330	None	775	?	220	None	77	None?	3.0	10	0.47	30	3.8	15
Dried: roller	3.0	25.0	27.5	37.5	0.91	1150	None	15	None	290	15	1150	None	2700	?	760	?	265	None	10.0	10	1.60	30	13.0	30
spray	3.0	25.0	27.5	37.5	0.91	1150	None	15	None	310	10	1150	None	2700	?	760	?	265	None	10.0	10	1.60	30	13.0	20
Skim milk																									
Raw	90.8	3.4	0.1	4.9	0.12	4		0		47	40	145	‡	360	?	103	?	37	60	1.5	10	0.30	90	2.0	60
Evaporated†	80.0	7.4	0.2	10.7	0.26	8	None	0		61		315	None	780		225		32		2.9		<0.10		1.7	
Sweetened condensed	29.0	9.6	0.3	58.8	0.34	12	None	0		120	10	410	None	1000	?	290	None	104	None?	3.8	10	0.60	30	4.8	15
Dried	3.0	36.0	1.0	50.5	1.26	40	None	1	None	450	10	1530	None	3800	?	1100	?	390	None	14.0	10	2.20	30	17.0	20

For simplicity, rounded-off values have been taken for raw milk and all other forms are assumed to have been derived from that particular milk. Two separate values are given for evaporated and for sweetened condensed whole milk to represent two different degrees of concentration current on the international market. ? Alone indicates possible slight loss. * Survival of vitamin C would depend on the amount originally present in the raw milk: 2 mg/100 g is for milk as it leaves the udder. † For milk prepared by the U.H.T. method and filled aseptically into cans (see p. 214) the losses would probably be of the same order as for U.H.T. sterilized or for sweetened condensed. ‡ Loss of riboflavin in the fat-globule membrane.

D in cod liver oil and in vitamin D tablets. Moreover, infant cereals are also enriched with vitamin D, and the use of proprietary vitamin preparations has been spreading.

OTHER FORTIFICATION. For particular purposes, for example for use in underdeveloped countries, it may be desirable to enrich dried skim milk with vitamin A in addition to vitamin D (13).

Apart from such reasonable schemes, there have been sporadic attempts to fortify milk with complex varieties of vitamins, minerals and other nutrients. In my opinion such ventures, unsupported by evidence of need or purpose, are wholly unnecessary and at times potentially dangerous.

Unintentional Additives

Oxygen. If milk as it leaves the udder is taken as the base line, changes due to the appearance of chemical substances in it occur even before any processing. Thus, as soon as milk is exposed to air, an extensive exchange of gases takes place, carbon dioxide being released into the atmosphere and oxygen being absorbed, so that when equilibrium is reached the carbon-dioxide content has fallen to two-thirds of its original value of 6.6 ml CO_2/100 ml and that of oxygen has increased fivefold to about 0.5 ml/100 ml (57). This oxygen later takes part in various oxidative changes that can affect nutritive value but may be largely avoided by proper handling.

Metals. The iron content of cow's milk as it leaves the udder is of the order of 0.3 ppm (23). Machine milking, cooling over metal coolers and subsequent storage in churns can easily almost double this figure. It is more important that, with worn tinned-copper utensils, the copper content of milk may rise considerably from the initial value of, say, 0.07 ppm (34). Because of the marked catalytic effects of copper in the oxidation of fat and formation of off-flavours, contamination with copper should be avoided at all stages of the handling of liquid milk.

Antibiotics. The presence in milk of residual antibiotics, largely derived from the treatment of mastitic udders, constitutes a growing problem of the dairy industry. The public health problems that may be associated with the presence of antibiotic residues in milk are: *1*) Exposure of the consumer to antibiotic-resistant udder pathogens that may be present in unpasteurized milk products. *2*) Development or aggravation of antibiotic hypersensitivity in consumers. *3*) Alteration of the intestinal flora of consumers, with possible reduction in vitamin synthesis (and absorption?). *4*) Alteration of the intestinal flora of consumers, permitting the growth and establishment of antibiotic-resistant strains of pathogenic microorganisms.

Jester *et al.* (35) expressed the opinion that "although antibiotics such as the tetracyclines, streptomycin, bacitracin, polymyxin, and neomycin, all of which may be components of mastitis preparations, should not be present in milk, their presence does not pose a public

health problem. . . . Penicillin is a highly active antigenic substance, and even the very small concentrations found in milk might well cause reactions in individuals highly sensitive to penicillin."

The presence of any antibiotic may affect the suitability of milk for the manufacture of certain milk products, and it is obviously desirable to eliminate them from market milk, but even more important to take steps to ensure the absence of penicillin from liquid milk.

Disinfectants and detergents. The influence of detergents and sanitizers on the quality of milk has been reviewed by Swartling (67). The trace amounts of these compounds that could go into the milk as a result of their normal use in sanitary production of milk are thought to be of little importance from the point of view of human health. Their normal and proper use in safeguarding the useful life of milk far outweighs any disadvantages. Chlorine compounds are unlikely to interfere with the properties of milk, but quaternary ammonium compounds have been shown to exert deleterious influence on acid formation in concentrations likely to occur in practice, unless the utensils are thoroughly rinsed. With all disinfectants it is important that precautions be taken to avoid intentional or unintentional addition of the compounds to the milk.

Insecticide residues. Synthetic organic insecticides in great variety are extensively used for the treatment of dairy cows and of barns in which they are kept. Traces appear in milk and, as some of the chemicals are highly toxic to man, constitute an evident health hazard. Marth and Ellickson (50–52) have authoritatively reviewed the problem. They found that in the U. S. A. a large proportion of samples from market milk supplies contained traces of chlorinated hydrocarbon insecticides, for some of which the Food and Drug Administration has set tolerance levels at zero ppm. Organic-phosphate insecticides are not secreted by cows into the milk even if ingested.

Radioactive fallout. The contamination of human diets with radiostrontium is posing the problem of increasing hazard to health. In those countries where consumption is high, milk is the chief dietary source of radiostrontium and the concern with this contamination is reflected in numerous publications, many of them quoted in a recent symposium of The Nutrition Society (58). The latest information about the position in the United States and Canada is given in (25) and about the United Kingdom in (1). Ways of removing radiostrontium from milk have been described (32, 53). Their possible use might be in accidents and calamities.

PROCESSING

General

In essence the purpose of processing is to keep milk palatable and safe until it reaches the consumer and there are many ways in which milk and its products can be prepared for the purpose. I propose to deal here only with those forms of milk in which the gross composition is unchanged or in which only fat or water or both have been removed or reduced. The values quoted in table 2 are largely based on findings at Shinfield. Where necessary, further details, and information about processes not studied there, are given.

Exposure to Light

In the first stages of handling even before processing, certain changes take place in milk. Some of them, connected with the entry of chemical substances, have already been mentioned. Another important one arises from the action of light. Exposure to it has three main effects. Firstly, riboflavin, a deep-yellow substance, absorbs visible light in the blue region and is destroyed by it. Thus, milk exposed to direct sunlight for 1 hour may lose, according to the intensity of the light, the temperature and the size of the bottle, 20–80% of its riboflavin (46). With careful handling the losses are very much less, probably not more than 3–6% (43, 46). Secondly, ascorbic acid which is a colourless substance and does not absorb, and by itself is not affected by, visible light, is oxidized in a photochemical reaction in which riboflavin acts as a sensitizer (cf. 38, 39). The reaction is in two stages. In the first stage, ascorbic acid is reversibly oxidized in the presence of oxygen to dehydroascorbic acid, still possessed of antiscorbutic properties. In the second stage, dehydroascorbic acid decomposes further even in the dark. The reaction is markedly speeded up by traces of copper and is very rapid in any heat process. The resulting products have no antiscorbutic activity. Ascorbic acid in the reduced form is relatively heat-stable and is not affected by pasteurization temperatures. Thus, the ascorbic acid content of commercial milk supplies varies greatly and they are not a reliable source of the vitamin. In the absence of oxygen and with little exposure to light, ascorbic acid in milk withstands most of the processing treatments.

The third important effect of exposure to light is the formation of taints. The sunlight flavour is now believed to be due to the formation of methional (β-methylmercaptopropionaldehyde), probably by the interaction of riboflavin with the amino acid methionine, though dehydroascorbic acid is perhaps also involved (60). A cardboardy flavour connected with oxidation of fat, and said to be due to unsaturated aldehydes (19) also appears in milk exposed to sunlight, particularly if it contains copper. This objectionable fault greatly affects the acceptibility of liquid milk, particularly by children.

Other factors play a part in the incidence of sunlight flavours, which are rare, for example, in England and were not evident last summer though it was exceptionally sunny.

Treatment by Irradiation

Ultraviolet light and ionizing radiations have been applied to milk as bactericidal agents for purposes of preservation.

'*Ultraviolet pasteurization.*' Exposure to ultraviolet light was used in the past on a large scale in the United States of America for increasing the vitamin D content of milk and is still so used in some German cities (45). The incidental effect of some of the German procedures is to reduce the bacterial count of the milk by some 70–80%. The Lembke apparatus developed in Germany is primarily intended for the pasteurization of milk though it may serve for the production of vitamin D milk as well (see 45). The side-effects of modern irradiation treatments are small and, apart from gaining vitamin D, the milk loses little in nutritive value, but as the method is not particularly satisfactory for either purpose its application is limited.

Sterilization by ionizing radiations. The preservation of milk through sterilization by ionizing radiations is for the time being of more theoretical than practical interest since, with liquid milk in particular, doses of radiation much smaller than those required for sterility produce off-flavours of sufficient intensity to make the milk unacceptable (29–31, 59, 68, 71). However, efforts are being made to prevent the formation of taints or to remove them (cf. 72). Dried milk is more resistant both to formation of off-flavours and to other radiation injury to nutrients (68). It should be emphasized that foods treated by ionizing radiations are neither toxic nor contain residual radioactivity (33); a diet containing 35% of irradiated dried whole milk and 65% of a nutritionally balanced synthetic ration proved nontoxic to rats in an 8-week feeding test (68).

In unpublished joint work with the Wantage Radiation Laboratories, Shinfield workers found that liquid milk exposed to 1 megarad lost two-thirds of its vitamin A and carotene, half of its vitamin E, 90% of its ascorbic acid and thiamine, some three-quarters of its riboflavin and 80% of its vitamin B_6. There was also some loss of nicotinic acid.

Heat Treatment

Liquid milk. PASTEURIZATION. *a*) The holder method (30 min at 61°–65°C). The effects are shown in table 2. In the commercial treatment milk loses some 20% of the ascorbic acid it contained on going into the plant. As discussed earlier, this latter quantity varies with the exposure of milk to light before processing and also with the extent of contamination with copper. For this reason pasteurized milk, as all other milks of commerce, is not a reliable source of the vitamin.

The loss in holder pasteurization of about 10% of the vitamin B_{12} observed by us (8) was at first sight surprising as the vitamin is relatively heat stable. Inquiry showed that ascorbic acid, sulphydryl compounds liberated by denaturation of milk proteins and glutathione may all be involved in the destruction of vitamin B_{12} that occurs when milk is heated (16). The destruction does not occur in the absence of oxygen, hence methods of processing involving deaeration are conservative of both ascorbic acid and vitamin B_{12}. Of the other vitamins studied, thiamine loses some 10% in holder pasteurization because of intrinsic lability. Apart from these changes, the nutritive properties of milk are not impaired and in particular the biological value of the proteins is not affected. Extensive feeding trials, carried out with children in England over 20 years ago by the Milk Nutrition Committee (54), form the best evidence for the equivalence of raw and holder-pasteurized milk in mixed diets.

b) High-temperature, short-time pasteurization (15 sec at 71°–73°C). In the variety of these processes the nutritional effects are even less than in holder pasteurization. In favourable circumstances, the direct loss of vitamin C need not exceed 5–10% and that of thiamine and vitamin B_{12} is below 10%. The same is probably true of the 'flash' process, in which milk is heated to 85°C for a very short time.

c) Pasteurized, deep-frozen milk. In a method developed at Shinfield (70) pasteurized milk is subjected to ultrasonic vibrations, is filled into plastic containers, frozen immediately and kept at temperatures not exceeding −12°C. Such milk can be stored for up to 18 months and on thawing has all the characteristics of freshly pasteurized milk, there being no separation of fat or precipitation of protein as is usual with ordinary frozen milk. Nutritionally, the milk is indistinguishable from freshly pasteurized milk (W. G. Wearmouth, private communication).

STERILIZATION. *a*) In-bottle sterilization. As used commercially in many countries for a long time, the process involves homogenization followed by heating at temperatures between 104° and 112°C for periods of from 20 min to 1 hour. The values given in table 2 show the effects as observed in England (8, 10, 24). Findings in the Netherlands are similar (11, 69). The process destroys at least half of the surviving vitamin C, about half the total vitamin B_6, some 30–40% of the thiamine and almost the whole of the vitamin B_{12}. It has no effect on vitamin A, carotene, riboflavin or nicotinic acid and its effects on the remaining vitamins studied is doubtful. The biological value of the proteins decreases by a few percentage units as a result of treatment (26), probably through slight inactivation or destruction of lysine (cf. 28).

b) Ultra-high-temperature sterilization (UHT treatment). These methods now coming into commercial use are based on a continuous process in which the milk is heated for a matter of seconds at temperatures between 135° and 150°C (21, 22). The results of tests on milk treated in one of such plants described by Burton (7) are given in table 2. It is evident that, as shown there, the effects are much less drastic than those of in-bottle sterilization. In fact they differ little from those of pasteurization, as testified by the relatively small destruction of vitamin B_{12} which, because of its great lability in processing, may be taken as a gauge of the severity of the treatment.

Uperization is probably equally conservative, though workers in Switzerland (4) found that it caused a

rather greater loss of thiamine than pasteurization. As would be expected from the inclusion of the deaeration process in the uperization treatment, vitamin C was largely protected and for the same reason there was no loss of vitamin B_{12} (17). It goes without saying that the more stable vitamins, A, D and riboflavin, are not affected. Workers in France (20) studied the Laguilharre process and detected no change in vitamin A, carotene, thiamine or ascorbic acid.

The UHT treatment has no effect on the biological value of proteins, as judged from experiments on rats (26) and thus the treatment can be taken as very conservative of the nutritive value of milk. However, in experiments at Shinfield with calves (66) UHT-treated milk proved decidedly inferior to pasteurized milk. The reasons are not yet clear, but may be connected with the denaturation in the process of some 70% of the non-casein protein fraction. It may be that the calf requires some essential components of the whey-protein fraction of milk. As will be shown later for dried milk, the calf is particularly sensitive to certain effects of processing and the bearing of these findings on problems of human nutrition is at present not clear.

In any event, unless the UHT process is coupled with aseptic bottling, which for the time being is practised only on a very small scale (2, 3), most of the UHT-treated milk must be re-sterilized in bottle before distribution. Such product is certainly no better than milk sterilized in bottle in the ordinary way (table 2).

Evaporated and condensed milks. Concentrated milk can be prepared in two main ways.

EVAPORATED MILK. In principle the preparation of evaporated milk consists in preheating the milk to about 95°C for some 10 min and then concentrating it in vacuum pans at a temperature around 50°–55°C, homogenizing the product and filling it into cans of suitable size which are then sterilized in a steam autoclave at about 115°C for not less than 15 min. In some recent processes the concentrated product is sterilized in continuous flow by UHT treatment and is then filled aseptically into cans (21, 22). The nutrient losses in the customary production are, broadly speaking, the same as in liquid milk sterilized in bottle. In the product treated after evaporation by the UHT method and then canned aseptically they would be expected to be of the same order as in UHT sterilized liquid milk (table 2). Recent work at Shinfield (10, 24) has shown that in conventionally evaporated milk the loss of vitamin B_6 is severe, 45–70%, and that a further 30% of the remainder is lost during storage for 6 months at room temperature.

SWEETENED CONDENSED MILK. In this product stability and bacteriological safety are achieved not by application of sterilizing temperatures but by addition of sucrose which acts bacteriostatically. The milk is preheated for some 15 min at 80°C before or after the addition of the requisite quantity of sugar, condensed under reduced pressure as in the manufacture of evaporated milk, and cooled in a manner to bring about crystallization of lactose in small crystals that do not cause grittiness. It is then sealed in cans or tubes. In modern modifications of the technique ultra-high temperatures are used for preheating, which helps to prevent the effect of 'age-thickening,' that is, solidification in the can in storage. Because of the less drastic heat treatment the nutrient losses are less than with conventionally evaporated milk and of the same order as in pasteurization (table 2). Sweetened condensed milk keeps well at moderate temperatures with no change in vitamin A, carotene or riboflavin, but with progressive loss of thiamine and vitamin C.

Dried milks. Microorganisms cannot multiply in the absence of water and its virtual removal enables milk to be kept for long periods. Freeze-drying of milk is not yet a commercial proposition for the enormous bulks to be handled, but drying by the application of heat has been successfully used for a long time. There are, broadly speaking, two distinct techniques. In roller-drying, preheated and sometimes homogenized milk is delivered in a thin film on the smooth surface of single or twin steam-heated rotating drums and the film of dried milk is scraped off with a knife as it forms. In its most modern forms, which may include pre-condensing the milk and enclosing the drums in a vacuum chamber, the roller process gives a high-quality product little damaged by heat. In spray-drying concentrated milk is 'atomized' into a current of hot air. The minute milk particles give off their moisture almost instantaneously and drop to the bottom of the drying chamber as tiny grains of dried milk. The modern tendency is to preheat the milk before spraying to at least 88°C for a very short time. The stability of powders so prepared, particularly with careful selection and hygienic preparation and control of the liquid milk, is such that they can be kept in air-pack for quite a considerable time at moderate temperature, there being no loss in palatibility, in the biological value of the proteins, or in vitamin A, carotene or vitamin C within 6–12 months. Otherwise gas-pack or vacuum-pack is necessary, and in any event air-tight containers are needed to prevent access of moisture to the hygroscopic powder. If the moisture exceeds 5%, various changes that set in on storage affect the solubility, palatability, colour and nutritive value, of which the most important is the so-called browning reaction in which the lysine of the protein combines with lactose and becomes biologically unavailable (43).

Dried skim milk, because of its low fat content, is much less prone than full-cream milk to oxidative changes and can be easily transported in bulk and stored, provided it is protected from moisture. It contains all the valuable nutrients of the non-fat milk solids, which are in fact concentrated in relation to whole milk because of the absence of fat that constitutes roughly one-third of the total milk solids. Being a by-product in the manufacture of butter or cream, dried skim milk is much cheaper than whole-milk powder and surpluses of it are available for export to, or distribution in, less well-nourished countries.

With high-quality powders produced in a modern

plant, the effects of drying, whether of whole or skim milk, need not be much greater than those of pasteurization, as shown in table 2 and as confirmed in experiments with calves at Shinfield (61–64) which showed distinct differences between powders produced by different methods.

Fink in Germany (14) found that rats given certain roller-dried milk powders as the main source of protein in low-protein diets developed liver necrosis, whereas rats similarly given fresh milk or certain spray-dried milks remained healthy. The latest information is that the necrosis can be prevented by small quantities of sodium selenite (15).

Instant milks. Even the best spray powders may be difficult to reconstitute owing to poor 'wetability.' The dairy industry in the U. S. A. has recently produced so-called instant non-fat milk powders which disperse easily and completely when added to water, the basis of the process being a change in the microstructure and porosity of the milk particles. Schulz (65) has given a good description of these products. Despite the double processing, such instant powders retain virtually unchanged the thiamine, riboflavin, nicotinic acid, pantothenic acid and vitamin B_6 of the fresh product, but some vitamin C may be lost in the second stage of the instant' process (27).

Soured and fermented milks. Fresh milk turns sour on keeping and this property has been used by man since the dawn of history for the preparation of countless palatable forms of soured or fermented milk, thus saving the milk from undesirable spoilage. There now exists a great variety of such local products, and in addition cultured milks, products rigidly controlled in manufacture and with a specific and well-defined flora, have achieved great importance in many countries with a well-developed dairy industry. Fuller information about these products and their nutrient value will be found in (42). It will be sufficient to say here that there is little tangible evidence for claims about special health-giving properties of soured milks, particularly those said to be associated with the establishment of a specific lactic-acid flora in the intestine. It is doubtful indeed whether any of these organisms, even those specially prepared for the purpose, can establish themselves and thrive in the gut. When it comes to the more strictly nutritive effects of fermentation, generalization is difficult because of the variety of treatments and conditions in which the fermented milks are made. The change of lactose into lactic acid or alcohol or both decreases slightly the calorie content of the product, by not more than 3 or 4% with the general run of sour milks. The vitamins survive quite well on the whole and though some proteolysis occurs in some types of fermentation, it is likely that the simpler protein fragments together with the unchanged protein have the same nutritive value as the original proteins of milk.

CONCLUSIONS

Heat treatment and processing are inherent in the provision of safe and palatable milk in its various forms. Certain additives may serve the same end and others, like vitamin D, enhance the value of milk for defined purposes. Any treatment involves some loss in nutritive value and unintentional additions, part of the price of modern civilization, may present some hazard to health. With modern methods of production intelligently applied nutritive losses are of little consequence when milk forms part of a reasonably planned mixed diet and detract little if at all from its importance as a protective food. The quality of milk as consumed depends not only on the treatment but also on the handling or mishandling to which it is exposed before and after processing. It is only by meticulous attention at all stages of its journey from the dairy animal to the table that milk can achieve and retain the quality made possible by modern technology.

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Nutritional aspects of processing and chemical additives on fats and oils

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OILS AND FATS are important human foods. They not only provide a concentrated form of the energy that people need to do their daily job, but they also include certain factors which are essential to life and which the human body does not synthesise. We have to ensure, therefore, that man's diet includes adequate amounts of fats, containing enough of the essential constituents.

Some oils and fats can be consumed in the form in which they are recovered from their natural sources. These include olive oil which is obtained by crushing ripe olives at the place of cultivation, and milk fat which is separated from fresh milk by centrifuging and is converted into its ultimate form as butter or ghee by churning and kneading and gentle heating. These products can be made in an appetising form, however, only if they are obtained fresh from the raw materials. The are available only in limited quantities. The same applies to beef tallow and lard which if freshly rendered, can be used without refining. But only a limited proportion of the two latter fats can be obtained in this way.

In most cases the raw materials for edible oils and fats are obtained in countries far away and the oils and fats cannot always be produced locally from the seeds or fruits owing to economic conditions. In cases where this is done—for instance with plantation palm oil—the taste of the fat prevents it from being used for direct consumption by the pampered Westerner. Moreover the quality of the product would deteriorate during transport over long distances. Coconut fat that could be obtained from fresh nuts in tropical countries has an attractive flavour but is not to everyone's taste.

In the vast majority of cases the oils and fats obtained from oil seeds and fruits contain substances that make them unsuitable for human consumption in that form, mainly because of their flavour or odour or appearance. Before becoming commercial products for human consumption, these oils and fats first have to be purified in order to remove the off-flavours, together with the colouring matter and free fatty acids.

On the other hand many of them have also to be processed to impart the required consistency to them. In some countries oil is used in the kitchen for the preparation of food. Other countries are more accustomed to using fats with a solid consistency, such as butter or margarine, or hard fats such as lard and beef tallow. Endeavours must therefore be made to process the raw materials so that they also will comply with the consumers' requirements regarding consistency.

It is the task of the oils and fats industry to process the raw materials made available to it by the world economy so as to make edible products which will guarantee a good diet and will suit living habits in different countries.

The main constituents of oils and fats are the triglycerides, esters of glycerol with a great variety of fatty acids. Many fatty acids can be synthesised by the human body; some, however, such as the essential fatty acids (cis-cis linoleic acid, arachidonic acid, etc.) cannot. Oils and fats also contain much smaller quantities of other constituents which are saponifiable and contain fatty acids as part of their molecules, such as phosphatides and waxes. In the natural oils and fats there are, moreover, unsaponifiable constituents such as hydrocarbons, e.g. squalene, sterols, e.g. phytosterols and cholesterol, and—in some of them—fat-soluble vitamins. Many oils and fats, especially the vegetable ones, contain vitamin E (tocopherol) which is practically all conserved during processing. Some contain vitamin A or carotene.

RAW MATERIALS

On the whole, oils and fats are obtained from animal fat tissue, from the flesh of oil fruits or from seeds. These cannot always be processed while fresh, and storage may lead to deterioration. This may be caused by bacteria and insects or by the effects of moisture, high temperature and atmospheric oxygen. These factors may in turn accelerate the effect upon the fat in fatty tissues of the fat-splitting and oxidising enzymes, the lipases and lipoxydases, which such tissue always contains. The result is that free fatty acids are formed on the one hand and mono- and diglycerides on the other, and further on some oxidation may take place. The better the raw materials are handled, the better the

quality of the crude oil will be and consequently of the refined oil.

Let us now discuss the main operations applied by the oils and fats industry one by one and see how far they influence the nutritive values of oils and fats.

PROCESSING

In order to separate the fat and to convert it into an edible form, we find that some of the following chemical and physical operations are necessary: *a*) separation from tissues, fruit or seed; *b*) degumming; *c*) neutralisation; *d*) bleaching; *e*) deodorising. Besides this the oils and fats industry applies other operations to make the fats more suitable for the purposes for which they are used. These are: *f*) hardening; *g*) esterification; *h*) mixing; cooling, kneading and—for special products such as margarine—emulsifying.

Separation of oils and fats. Before oils and fats are separated the tissue and the seeds must be finely divided in order that the fat released from the cells can run off properly. This is followed by heating which, in the case of animal tissue, already releases a substantial part of the fat. This is the process applied, for instance, in melting out beef tallow, lard and whale oil. Some fat remains which is absorbed in the cell walls and can later be separated by pressing or centrifuging. The same applies to the flesh of fruit, for instance palm fruit.

In the case of seeds, the fat is often more difficult to remove. After crushing, the material is heated and most of the fat is removed by pressing at high-pressure and temperatures of about 100°C.

Besides this process based on heating and pressing, extraction is often used to obtain a higher yield of oil. The extractant used nowadays is almost always a low-boiling petroleum fraction: usually technically pure hexane with a boiling point of about 66°C. Solvents used in the past, such as trichlorethylene, which has the advantage of being noninflammable, have been completely abandoned. The reason for this is that it brings into solution many more substances (nonfats) from the seeds which cause trouble during refining. Moreover trichlorethylene when brought into contact with water very easily forms hydrochloric acid, which may corrode the steel of the apparatus.

For economic reasons a combination of expelling and extraction is often applied. A fairly high oil content remains in the pressed seed which is then crushed again and solvent-extracted. After extraction a meal is left with a low oil content (0.5–1.0%) and a solution of oil in solvent, the miscella. From this miscella the solvent is distilled off and finally volatilised by blowing through steam.

It has sometimes been thought that the solvent might contain higher aromates having a toxic effect. Extraction solvent is so well fractionated, however, that there will certainly be no higher fractions in it. After refining the edible oil is completely free from solvent. This has been proved, inter alia, by using extracting agents containing radioactive tracers. After the solvent had been distilled off and after neutralisation, washing and drying under vacuum at 90°C, all the radioactivity had vanished.

Degumming. When the oils and fats are removed from their natural sources (tissues, seeds) some constituents other than triglycerides are also partly dissolved in the oil. In many cases it is useful to remove such substances, e.g. phospholipids, before further refining is commenced. This applies particularly to soybean oil, for instance, in which quantities of phospholipids amounting to several per cent are often found after extraction from the beans. These can easily be removed by swelling the phospholipids with small quantities of water, after which they can be separated by centrifuging. This product is the well-known soya-lecithin. For degumming, chemical agents are also often used, for instance sodium chloride solutions, phosphoric acid, citric acid, acetic acid, acetic acid anhydride, hydrochloric acid, etc. It has been found that many of these substances are separated more easily under the influence of these agents. This degumming usually takes place at temperatures below 100°C. The agents do not appear in the degummed oil and the nutritional value is not affected by this operation.

The phosphatides that are removed may be important food constituents. Their presence makes an adequate refining into tasteless fats impossible, but the lecithin may be purified separately and subsequently re-added to margarine to improve the frying properties. It is also used to improve the viscosity of chocolate in manufacture.

Neutralisation. As I have already mentioned, some splitting has occurred in the oils and fats through storing the seed or the fatty tissue under the influence of moisture and lipases, as a result of which free fatty acids and mono- and diglycerides have been formed from the triglycerides originally present. Although in many cases, i.e. with long-chain acids (C_{16} and higher) these fatty acids do not adversely affect the taste of the oil, it is advisable to remove them, as they affect refining of the oil and the use of the final product (low smoke point).

The fatty acids combined with caustic soda in solution to form soaps are removed as an aqueous phase that settles at the bottom of the neutraliser vessel and can be drawn off or removed by centrifuging. This alkaline aqueous phase also contains part of the colouring matter and other unwanted substances. This is an additional advantage of the neutralisation process, as the latter might adversely influence the taste of the oils and fats in further processing. Remains of the soap phase are removed as well as possible by washing with water. This entire operation which is carried out at temperatures below 100°C definitely does not remove any integral constituent of the diet.

Bleaching. After the fatty acids have been neutralised, we have to remove as much as possible of the colouring material that is still present. Strongly coloured oils are difficult to make tasteless. At the same time the bleaching

process eliminates the soap residues left in the oil after neutralisation and washing with water.

This bleaching is done by treating the oils and fats under vacuum with bleaching earth at a temperature of 90°–100°C. Some fats, such as coconut- and palm-kernel oil, are treated with small quantities of activated carbon in addition to bleaching earth. The decolouring is for the greater part a purely adsorptive action at the surface of bleaching earth and activated carbon.

In addition, however, slight changes may occur in unsaturated oils and fats under the influence of the bleaching earth. The extent to which this takes place depends partly upon the past history of the crude materials.

Mitchell and Kraybill (8) first observed that under the influence of bleaching earth characteristic changes occur in the ultraviolet spectra of edible oils. Absorption bands develop at 268 mμ when the oil contains linoleic acid and 268, 300 and 316 mμ when linolenic acid is also present. These absorption bands indicate the presence of conjugated trienes or tetraenes, which are formed only when the oil has been previously oxidised. The trienes are formed from primary hydroxy fatty acids or hydroperoxides by the splitting off of water under the influence of bleaching earth. The absolute concentration of these trienes and tetraenes is however very small, viz. about 0.1 %.

Polymerisation may also occur under the influence of bleaching earth, especially in oils with a high unsaturated fatty acid content. The normal test on polymers in oil (Hügel test) is negative, but more sensitive methods for detecting dimers have taught us that while the content in crude oil is 0–0.01 % it goes up to 0.01–0.08 % in neutralised and bleached soybean oil, to 0.02 % in sunflowerseed oil, to 0.03 % in cottonseed oil and to 0.02 % in rapeseed oil.

Nevertheless these reactions occur to such a small extent and the compounds are of such a kind that no influence on the food value is to be expected from them.

Deodorising. After gums, fatty acids and colouring material have been removed, it is necessary to eliminate the off-odours and flavours from the crude oils and fats. The usual method that has been applied for many years is steaming under vacuum. The oil is pumped into vacuum containers, heated to 160°–230°C (normally 180°C) under a constant stream of superheated steam which entrains the odoriferous substances. This method can be effected batchwise, semicontinuously or continuously and its big advantage is that treatment under high vacuum prevents oxidation, which might lead again again to deterioration of the refined oil. The steaming time and the quantity of steam passed through are determined by the taste, the operation being continued until the fat is tasteless.

During the deodorisation process some chemical reactions can take place under the influence of steam and high temperature. Some saponification will occur through which free fatty acids will be formed that are distilled off. In addition to this, heating may also cause polymerisation. The same sensitive methods as mentioned before have shown that steaming has only very little effect on the formation of polymers.

Deodorisation of sunflowerseed oil at 240°C, a temperature which is not applied normally (180°C) increased the dimer content from 0.02 % to 0.07 % and 0.08 %. With soybean oil the dimer content when deodorised at 170°C increased from 0.05 % to 0.08 % and at 235°C from 0.05 % to 0.10 %. In groundnut oil and hardened groundnut oil the dimer content was increased by bleaching and deodorisation from 0.01 % to 0.06 %.

Experiments so far have not indicated even the slightest effect upon the growth of rats attributable to these extremely small quantities of dimers. Only if polymerisation is increased up to contents of 10 % and 20 % in the treated oil is growth affected, because the highly polymerised portions are no longer digested and pass out of the body again without having been used.

Considering all the processes we have discussed so far, i.e. degumming, neutralising, bleaching and deodorising, we can say that the fat that is obtained has in all likelihood the same nutritional value as the starting product, but has become more palatable. As to the latter, our views will hardly differ. To elucidate what I have said regarding nutritional value, let me mention comparative experiments we made with rats.

These animals had been fed diets with a high content (50–70 cal. %) of an unrefined fat; as controls served groups which were fed comparable amounts of the same, but refined fats. According to Thomasson these diets with an extremely high fat content give an excellent opportunity of detecting even slight differences in growth stimulation between various fats. At the levels normally used in human diets these differences will be much smaller, or even vanish (cf. table 1).

A number of other oils and fats are still being investigated. The results of these tests will be known in September 1960.

Hydrogenation. In addition to the treatments outlined above, hydrogenation methods are also applied in the edible oil industry in order to meet the requirement that for satisfactory usability many fats must have a solid consistency. Of course it is possible to use solid fats, but these are often in short supply. Moreover such products frequently do not have the required melting

TABLE 1. *Growth on Crude and Refined Fats in Rat Tests*

	Growth of Animals				
	1 Wk.	2 Wk.	3 Wk.	4 Wk.	5 and 6 Wk.
Crude olive oil, 50 cal.%	26.3	64.9	101.4	135.3	
Refined olive oil, 50 cal.%	26.9	65.8	102.9	140.7	
Butterfat, 70 cal.%	15.1	41.3	78.8	119.2	160.2
Refined butterfat, 70 cal.%	15.6	40.1	72.1	109.9	148.9

behaviour and consistency to come up to the climatic conditions of the country in which they are used. For this reason, it is frequently necessary to harden oils, and sometimes, although to a smaller extent, also solid fats, in order to impart the desired consistency to them. In addition, fish oil and whale oil, which as such are unsuitable for human consumption, must be hardened. In this process the highly unsaturated fatty acids which can give an unpleasant fishy taste, are converted into dietary fats containing fatty acids of satisfactory taste. Of the total quantity of oils and fats used in the edible oil industry about 30% is subjected to this treatment.

Before they can be hardened, the crude oils and fats are first neutralised to remove fatty acids and mucilage which unfavourably affect the hardening. The mucilage and other catalyst poisons, possibly sulphur compounds, could inactivate the catalyst, while fatty acids are able to dissolve the nickel present in the catalyst.

After the addition of an amount of suitable nickel catalyst, the product is hardened at temperatures from 100° to 200°C, while hydrogen is passed through, until it has required the desired properties. By the nature of the catalyst and the hardening method, the properties of the final product can, within certain limits, be controlled. The height of the melting point of the fat and its dilatation values at different temperatures are generally taken as criteria for the final result.

As a result of this hardening, which in the case of edible oils and fats only removes part of the unsaturation, various changes take place in the fatty acid molecules. A part of the double bonds in the fatty acids is saturated. At the same time a shifting of double bonds in the molecule occurs, accompanied by a transformation of a part of these double bonds from the cis- into the trans-form.

Hardened fats have been investigated in order to find out in what way their nutritive value is related to that of unhardened fats.

During hardening a minute amount of nickel is dissolved; but by careful refining (neutralisation, bleaching, deodorisation) of the hardened fat, the dissolved nickel is again removed. As a result, hardened fats contain extremely small amounts of nickel (1–4 μg/kg), much lower than are found in many natural products (100–300 μg/kg).

From investigations by Aaes Jörgensen *et al.* (1–5) and by Thomasson (11, p. 446 *et seq.*) it has appeared that on feeding rats diets containing a high percentage of hardened fats, there is some slight decrease in growth rate as compared with the unhardened products. However, Thomasson has further shown that diets containing hardened oils are completely satisfactory and support normal growth subject to the presence of 3–4% of linoleic acid in the diet. He has further shown that for satisfactory growth it is necessary to have a proper balance between saturated fatty acids with 12–18 carbon atoms and linoleic acid.

It has been investigated whether the trans-fatty acids formed from natural cis-fatty acids have identical nutritive values as the original acids. Sherman *et al.* (9) have published in 1949 some work on the influence of elaidic acid compared with oleic acid and petroselinelaidic acid compared with petroselinic acid. The cis-acids gave some better growth and a higher arachidonic acid content in the body fat. Different results were obtained by Thomasson (11, pp. 471, 472), who has compared a fatty acid having a cis-double bond (oleic acid) and the corresponding fatty acid having a trans-double bond (elaidic acid). It was found that even a very high dosage of elaidic acid gave normal growth, and no indications were obtained that the depotfat-formation and the combustion proceeded differently from those of normal oleic acid. In addition the growth on feeding elaidic acid was not changed by the addition of linoleic acid. As is generally known, trans-fatty acids are also to be found in butter and beef fat.

Thomasson established that on admixture of unhardened and hardened oils, a growth was obtained which in most cases exceeded that of the original non-hardened oil. This assures us that no harmful substances are formed during the hardening process.

From the results of numerous investigations it is at present assumed that a high content of unsaturated compounds may have a blood-cholesterol lowering effect. This is actually a fact which has to be taken into account. For this reason it would seem advisable to see to it that during the hardening the content of unsaturated fatty acids decreases as little as possible. The theory that in this respect the essential fatty acids are important, now appears to be incorrect. Various oils (whale oil, fish oils and rapeseed oil) which have a low content of essential fatty acids are, according to Ahrens, Brock, Malmros and Thomasson, more effective in bringing about a decrease in the blood-cholesterol content than oils having a very high content of essential fatty acids.

Moreover Thomasson is investigating the influence of various fats and fatty acids on the blood-cholesterol content by means of a rat-screening test. He has found that by proper hardening of various oils and fats, marine oils, cottonseed oil, soybean oil, etc., provided this process is not continued to too high melting points (not in excess of 35°C), there is very little effect on the cholesterol-lowering action. In addition it was found that not only the degree and the nature of unsaturation is important, but other factors may play a role as well. According to Thomasson it is probable that the longer the chain length of the unsaturated fatty acid, the greater the effect will be on blood-cholesterol lowering. These experiments are only in the initial stage and will need confirmation on human panels.

In all the discussions on the influence of specific fats on growth, blood-cholesterol, etc., it must, however, be kept in mind that man seldom consumes a single fat. Indeed, we eat much fat in such products as fish, meat, cereals, etc. This invisible fat comprises 40% of the total fat intake in some Western countries and up to 70% of the fat intake in certain underdeveloped coun-

tries. Thus the human diet generally contains a mixture of many kinds of fat, which individually may have a specific action. This, however, will be modified when in admixture with other fats.

It is perhaps useful in conclusion to consider the contribution that hardening has made to the fat industry. As I have observed already, the advantage of hardening is that with the prevailing shortage of solid fats, a product suitable for certain climatic conditions can also readily be made from oils. Next, the decrease in the degree of unsaturation imparts better keeping qualities to the product. When the degree of unsaturation is higher, the fat is usually more susceptible to oxidation. This means a shorter shelf-life and increase in the chance that oxidised fatty materials which are not always harmless will be consumed. It is important, therefore, to find a compromise between these various factors.

Esterification. To improve the consistency, and to impart other desired properties to margarine, shortenings, and similar products, interesterification is frequently applied. In this process a fat or a mixture of fats is treated with a catalyst which is able to rearrange the fatty acid-radicals in the glyceride molecules via a transition stage, in such a way that a random esterified product is obtained. In addition, by application of so-called directed interesterification, high-melting triglycerides formed at low temperature can be made to crystallise, thus effecting a fractionation at the same time. In this operation catalysts are employed (sodium, sodium glycerate, sodium methylate and sodium ethylate) at temperatures varying from 50° to 150°C. Conditional for this operation is that it is carried out with a completely dry fat, as otherwise the catalyst is split by the water present, before the interesterification reaction has taken place. In this operation, the fatty acids undergo no change whatever. Only their position on the glycerin molecules changes. These conversions are comparable with the processes going on in the animal body during fat metabolism, when partial splitting off of fatty acids and subsequent re-synthesis to fats of frequently quite different compositions take place.

In addition to interesterification it is also possible to re-esterify fatty acids as such or a mixture of fatty acids and glycerides to a triglyceride. If in this process the starting products are fatty acids originating from natural fats either of sufficient purity or which have been purified, for instance by distillation *in vacuo* of the relevant fatty acids, no objections can be raised to this procedure on physiological grounds. These acids can then be re-esterified with glycerin in various ways, under the influence of catalysts. This process was formerly carried out at high temperatures, while using, inter alia, tin as a catalyst. Now it is, however, also possible to carry out this esterification at much lower temperatures.

The interesterification process does not affect the nutritive value of a fat. This is especially clear from the results of relevant experiments. Thomasson carried out tests with rats; the growth of the animals was determined after administration of diets containing high percentages of triglycerides in their original state by the side of the fatty acids made from these triglycerides and then re-esterified. The results can be seen in table 2. No significant growth difference could be found.

TABLE 2. *Growth of Rats Fed on Diets Containing Natural Triglycerides and Re-esterified Glycerides Made From the Same Fatty Acids*

	Calorie Percentage Fat in Diet	Growth
		after 3 wk
Hardened groundnut oil	72	41·5 g
Fatty acids re-esterified	72	39·0 g
Low melting fraction f.a. re-esterified	72	44·5 g
High melting fraction f.a. re-esterified	72	41·0 g
		after 4 wk
Groundnut oil	50	131 g
Groundnut fatty acid distilled re-esterified	50	123 g

Physical treatments. In addition to the methods discussed so far, and which are all to a greater or lesser extent based on chemical reactions, various physical treatments are likewise employed in the edible oil industry. First among these is the mixing of various oils and fats as is current practice in the edible fat industry. In the margarine industry, for instance, oils, solid natural fats and hardened oils and fats are mixed until a composition is obtained which has a satisfactory consistency for the climate and the use for which the product is intended. The same applies to nonwater containing edible fats. This mixing of various fats is not only advantageous from the point of view of use, but it may also favourably affect the nutritive value of the mixture.

From the work by Thomasson we have learnt that on feeding pure fats in diets with a high fat content to rats, a fluctuating growth is observed in the test animals. The greatest growth-action is found in the case of fats such as lard, butterfat, olive oil, cottonseed oil, and sheafat. Beef tallow, maize oil, soybean oil, groundnut oil, and palm oil, at a level of 70 cal. %, give a slightly less marked growth, while other fats such as sunflowerseed oil, coconut fat, sesame oil, whale oil, herring oil, and rapeseed oil, when amounts of 50–60 cal. % are contained in the diets, give even less good growth (10). On mixing various fats these differences can be levelled out, and so give a product which has a more uniform growth action. This brings me to the point I made earlier when I stressed the nutritional advantages of diets containing mixed fats. The range of both visible and invisible fats in our diet normally ensures this.

It is clear that the physical process of mixing introduces no changes at all in the nutritive properties of each of the component fats. The same applies to other physical treatments which are used in industry, such as the emulsification process in the margarine industry where skimmed milk is emulsified in a fat phase, the cooling of fat mixtures on cooling drums or in votators, the sub-

sequent kneading of the cooled fat in various types of kneaders.

ADDITIVES

In the foregoing pages the additives used during the processing of oils and fats and their possible influence on these raw materials have been described in great detail. Now some attention will be paid to those additives which are added to the refined fat or fat mixture.

Vitamins. Low concentrations of fat-soluble vitamins are found in oils and fats. As already mentioned in most vegetable fats vitamin E is present, of which only a small portion is lost during refining. In animal fats only a minute amount of this vitamin is present. Beef tallow contains small amounts of vitamin A; butterfat contains a larger amount of vitamin A; a large amount of carotene is found in palm oil. Vitamin D occurs in butter in very small amounts.

During refining of fats and oils the greater part of the carotene and of the vitamin A is lost. It would be possible to retain part of the carotene in palm oil by application of a special refining technique, but the taste of palm oil so treated becomes poor due to the presence of decomposition products originating from carotene and similar colorants. Therefore if palm oil is used in large amounts, it has to be bleached. It is possible to recover the carotene contained in palm oil, and suitable processes have been worked out. Currently produced synthetic β-carotene, however, is completely pure and its price is not any higher than that obtained from palm oil by applying the methods known at present.

To meet the vitamins A and D requirements of the population, synthetic vitamin A and synthetic vitamin D are added to a number of fat products, inter alia margarine. The amount of added vitamin D must not be too high, for instance *ca.* 31U/g, to prevent the danger of giving older people an over-dose of this vitamin.

Flavouring substances. Attempts are being made to impart an attractive taste to a number of oils and fat products, notably margarine. This is done by the addition of synthetic flavouring substances. For a long time past diacetyl has been used for this purpose; at present also lactones and other flavouring substances are employed. Physiological tests have shown that these substances, even when added in very large amounts to the diet, are harmless for the animal organism. This has been proved in short-term experiments and in tests covering the entire life-span of rats fed these substances. At a later date it has moreover appeared that these substances are identical to the flavouring substances found in butter.

Anti-oxidants. To improve the shelf-life of edible oils fat-soluble anti-oxidants could be used. As a rule it can be said that these substances have little effect on vegetable oils, but a pronounced action on being added to animal oils and fats. These latter products contain only small amounts of natural anti-oxidants, so that they can be markedly improved by the addition of natural tocopherol or such synthetic anti-oxidants as gallates (propyl, octyl and dodecyl), nordihydroguaiaretic acid (NDGA), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT). Vegetable oils and fats contain natural antioxidants, inter alia tocopherol. Especially γ-tocopherol is active as such at room temperature. It has appeared that during refining and hardening of vegetable oils only a small part (10–20%) of the tocopherol is converted. Only in those cases when a vegetable fat must be used in edible products which have to be stored for very long periods of time, e.g. biscuits for export, it will be advantageous to add an anti-oxidant to a vegetable fat.

The above-mentioned anti-oxidants have all been tested biologically, and found to be harmless. It is not necessary here to go into details concerning all the experiments which have been carried out, but they form the basis on which the use of these anti-oxidants has been sanctioned in various countries.

Anti-microbial substances. In some water-containing fat products, such as for instance margarine, the use of preservatives is allowed in many countries. These products have the advantage that even in nonsalted margarine a large amount of milk can be used, without the danger of rapid deterioration. As such especially benzoic acid and sorbic acid are being used. These two acids, as has been proved by biological investigations, can be considered harmless to health. Sorbic acid, however, because it is decomposed in the normal way in the body, is somewhat preferred to benzoic acid.

Colouring matter. The colorants currently used in oils and fats are generally synthetic carotene, palm oil and annatto. In a number of countries fat-soluble aniline-dyes are still allowed, but their use is gradually abandoned, mainly because to fat-soluble aniline-dyes a more unfavourable effect on the animal organism is ascribed than to water-soluble aniline-dyes.

Synthetic carotene which is identical to natural β-carotene (pro-vitamin A) has been discussed higher up. Annatto, the coluring constituent of which, bixine, is present on the outside of the annatto seeds, can be used without any harmful effects on health (6, 7). The annatto seeds are procured in Central America where they are used on a large scale in the preparation of food. The colouring material is dissolved in oil, and this oil can be added to edible fats.

Emulsifiers. The most important emulsifiers used in the oil and fat industry are: MONO/DIGLYCERIDES. At low temperature these substances have the tendency to retain the water phase finely dispersed in the fat phase, which is especially advantageous in the margarine industry. These mono- and diglycerides are preferably prepared from hardened fat, because in that case they have better action than when mono- and diglycerides from lower-melting fats are added. From the work by Frazer and other research-workers it has appeared that in man, on consuming fats and oils, part of the triglycerides is broken down to mono- and diglycerides. Moreover, mono- and diglycerides are present in

natural and also in refined oils and fats. Consequently, they form normal constituents of natural oils and fats and normal degradation products of fat metabolism. Their value as foodstuffs is consequently the same as of triglycerides.

LECITHIN. As already mentioned under desliming, a phosphatide is obtained from a number of oils, notably soybean oil, during this treatment. After purification, this phosphatide can be used to improve the frying properties of products such as margarine. Moreover, lecithin is a substance known to have favourable physiological effects.

SCIENTIFIC RESEARCH

The oils and fats industry, like many other industries, originally developed almost entirely along empirical lines. Some processes, such as neutralising and deodorising, were originally applied in a comparatively primitive fashion, but grew up through their practical application during the course of years.

The process of hardening, however, had a somewhat different starting point. This process is in fact based on scientific research and was initially due to the work of Sabatier and Senderens who hydrogenated unsaturated compounds in the gaseous state using catalysts. Their method was later applied by Normann to liquid oils and fats. The hardening process and the catalysts employed were further improved, mainly by trial and error methods, but nevertheless with striking results.

It is only in the last few decades that research laboratories in the modern sense of the term have begun to play an important part in the industry. Scientific means were sought to study the effectiveness of various processes and for the development of means to improve them. A desire for perfection ensued and progressive advancement has lead to the present high state of development.

A stage has now been reached at which further improvement of the various processes employed is only likely to be achieved through more fundamental work. The direction in which further improvements can be sought can only be seen when the fundamental processes involved are better understood. In hardening, e.g. x-ray, work is being undertaken to determine the crystal size in nickel catalysts, which in turn should provide a better insight into the mode of action of the catalysts. New techniques, such as spectrophotometry, gas chromatography and mass spectrometry, etc., are being generally applied in the oils and fats field and have already cast new light on many of the problems of the chemistry and physical nature of these substances.

From this account it must be abundantly evident that in addition to chemical and physical research an important position is occupied by biological research. Great attention is being devoted to the possible physiological influence of dietary oils and fats. Much of this research is being undertaken in cooperation with working groups in universities, but it is yet too early to say what the consequences might be. It is certain, however, that in this way industry can make an important contribution to the development of science and to public health.

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Modern technology as related to the safety of foods

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FACED WITH THE VAGARIES of climate and rainfall and the uncertainties of hunting and fishing, primitive man had to learn how to preserve his hard-won food. Thus it came about that methods of drying, salting and smoking were discovered. These basic procedures, crude indeed by modern standards, are essentially the same as those employed in modern food processing.

Pickling, spicing, and protection from air by covering with brine, sugar, sirup, or fat, were among the more sophisticated processes developed by primeval and medieval man. Preservation by heating and deaeration, though known for many centuries, received its major impetus with the invention of the canning process by Nicholas Appert in France, and its development by him and his contemporaries, Thomas Saddington and Bryan Donkin in England.

Knowledge of the basic mechanisms involved in food preservation was largely empirical. Appert's method of hermetic sealing was predicated on the belief that putrefaction was caused by exposure to air. It was not until the significance of Louis Pasteur's germ theory was fully appreciated that a rational explanation could be advanced for the modus operandi of heat processing, as well as for many of the traditional forms of food preservation. It was Pasteur's contributions, perhaps more than any other single factor, that led to the development of food technology as we know it today. In the century that has elapsed since his discoveries, the effects of temperature, moisture, air, and chemical milieu on the growth of microorganisms, and collaterally on enzyme action, have been exhaustively studied. This knowledge has not only provided explanations for the traditional methods of food preservation, but has given rise to variations, refinements, and improvements in technology introduced in recent times.

In view of current popular misconceptions propagated in certain quarters, to the effect that food processing, in devious ways, is directed toward making stale foods appear fresher, or inferior foods appear better, it may be profitable to consider how social and industrial progress have influenced food habits, and hence food technology. The development of world-wide navigation in the 15th and 16th centuries was stimulated in part, at least, by the search for easier routes to lands where spices were grown. But more than that, the long voyages on which ships embarked necessitated the preservation of the food supplies and of the foods brought back from the distant shores. Incidentally it was not until hundreds of years later that it was realized that lemons contain a specific against sea-scurvy or that spices contain antioxidants and antimicrobial agents. When transportation by rail became the order of the day, migration occurred from farms and villages to towns and cities where the population became increasingly dependent on foods raised and transported by others than ultimate consumers. Movement of food from farm to home has even to this day imposed special requirements on food processing by reason of *a*) sanitary and health considerations of which we have become more cognizant since the advent of the science of bacteriology; *b*) economic considerations related to the cost of shipping, storing and handling, down to the retail level; and *c*) changing social customs and patterns of living, such as smaller living quarters, job-holding housewives, the vanishing tribe of cooks and servants, and the car for every home. The tremendous increase in food packaging, which developed from the foregoing considerations, and the concomitant legal requirements for labeling, which identified the manufacturer and promoted the use of brand names, made it essential that food products meet, and consistently maintain, certain standards of quality and uniformity. Suffice it to say, that in food production, processing and distribution, as in many other facets of life, *altera tempora altera mores.*

To revert to the main subject, viz. the relation of technology to the safety of foods, let us consider the scope of modern processing techniques in contrast with basic, time-honored methods. Most of the operations used in food processing entail the application of one form or another of energy, chiefly thermal or mechanical. For example the application of heat in such culinary operations as blanching, boiling, baking, broiling, roasting, and frying are the major processing steps as they have always been. However, nowadays the objective is not merely preparative, but preservative, and the conditions are carefully controlled to effect sterilization, pasteurization or other desired objectives with a minimum of adverse change in nutritional or organoleptic quality. Modern food technology is, in fact, characterized throughout by the increasing use of quality control methods. Moreover the conditions for heat processing are constantly being improved to permit optimum reten-

tion of flavor, texture and similar esthetic qualities, compatible with safety. Examples are the use of high-temperature, short-time processing, and of aseptic filling in modern canning operations.

Many of the fermentation steps used from time immemorial in the production of bread, cheese, beer, wine, vinegar, and various pickled products, are now rigidly controlled by means of known additions of enzymes, microbial cultures, and buffers, and the maintenance of uniform physical conditions. Many new enzyme products and special uses for enzymes have been developed such as the in situ production of invert sugar in confectionery, the clarification of fruit beverages, the maintenance of whiteness in dried egg albumen and the decomposition of excess hydrogen peroxide used for bleaching purposes.

Among the most ancient food processing operations were the milling of cereals and the grinding of spices. These together with fermented beverages were incidentally the most easily, and hence the most frequently, adulterated foods. The development of analytical chemistry and microscopy during the last century marked the beginning of the end of such gross practices. This year we are celebrating the 100th anniversary of the adoption of the English food laws. Today milling consists not merely grinding but involves sifting, flotation, and other physical separations, carefully designed to yield numerous products from wheat, corn, and other grains, with a variety of nutritional, organoleptic, functional, and keeping properties. Some of these products entail the use of chemicals to restore or stabilize nutrients, to standardize leavening properties, or to improve the appearance and texture of the end product.

The aging of flour to facilitate the handling of dough in modern bread-making machinery and to maintain uniform loaf texture, is now accomplished by chemically controlled maturation. Many products derived from cereals and grains are subjected to mixing operations that involve extreme exposure to air, or to rolling, flaking, puffing, toasting, pressing and pelleting operations that cause elevation of temperature, either deliberately or incidentally to the application of pressure. The effects of heat and aeration on the nutritional and safety aspects of foods must be considered in connection with the concentration, evaporation, and spray or roller drying of milk, fruit juices, and other beverages.

Aside from canning, one of the most widely employed commercial processes for treating fruits and vegetables is dehydration. In place of the more primitive method of sun- or hot-air drying, this is now effected by exposure in trays or on moving belts under vacuum or in an inert gas, e.g. nitrogen or the gases resulting from the controlled combustion of natural fuels. In order to prevent darkening of certain fruits, advantage is taken of the antioxidant effect of sulfur dioxide, ascorbic acid and erythorbic (isoascorbic) acid.

Certain processing operations are directed to the separation, isolation or concentration of various fractions of naturally occurring products. For example, peeling, sorting, screening, straining, filtration, deionization, centrifugation, extraction and distillation are aimed toward separating wanted from unwanted components of foods. To the extent that they involve the removal or leaching of vitamins or minerals, as may occur in the conveyance of fruits and vegetables through flumes or by flotation or washing, or the actual destruction of vitamins by excessive exposure to air, light, or heat, these operations may influence the nutritive value of the final foods.

In the refining, deodorizing, and hydrogenation of edible oils, high temperatures are employed. However this is done under vacuum or in an inert atmosphere (e.g. in steam under reduced pressure) thus minimizing oxidative destruction. These operations may nevertheless affect the natural antioxidant content of vegetable oils and may cause heat-polymerization. The latter is also a factor of importance in the processes of deep-fat frying of potatoes, fish, snack foods, etc.

In any discussion of the manifold operations used in food processing, mention must be made of refrigeration, particularly in its more modern aspects involving subfreezing temperatures, the combination of freezing with drying or with antibiotics, and special conditions associated with packaging and distribution of frozen foods. These are not generally regarded as susceptible to nutritional deterioration or to changes of a hazardous nature. But when it is remembered that, except when precooked, frozen foods are not usually subjected to preliminary heat sterilization, and that they are exposed to thawing either in the channels of commerce or prior to use in the home, the possibility of bacterial spoilage, even though slight, cannot be ignored.

One of the major innovations in the field of food preservation in our time has been the use, or rather the potential use, of ionizing radiations and high speed electron beams. This has been experimentally investigated most intensively from the standpoint of both functional practicability and potential hazard. Among the possible applications are the prevention of sprouting of potatoes, the destruction of insects in grains, cereals, and spices, as well as the in-package sterilization of raw meat and fish. Whereas present indications show promise of limited usefulness of radiation as a pasteurization technique, either alone or in conjunction with other more conventional methods, serious limitations with respect to off-flavor development, particularly in the case of complete sterilization of meats, remain to be overcome. The higher radiation dosages required to inhibit enzymic, in contrast with microbial, deterioration may play a crucial role in determining the future of this means of preservation.

Lastly, but possibly of greatest current interest in relation to the safety of foods, are the large number of chemical substances whose numerous uses in food processing have only begun to be realized recently. (For the purpose of this discussion the term 'food additive' will be used in its broadest aspect rather than as defined in the Federal Food, Drug, and Cosmetic Act (3), thus including any chemical substance present in food as a result of its use

in production, processing or packaging technology.) On the one hand a food additive may be present as an unintentional residue from the preharvest application of a pesticide to a plant, or from the administration of a drug to a meat- or milk-producing animal or it may be present incidentally from its use as a processing aid or from contact with processing equipment or packaging material. On the other hand, it may be intentionally added to impart a desired quality to the end product in which it is expected to remain. Regardless of whether or not it is purposefully present, the question of safety of an additive in the amount present and under the conditions of intended use of the food must be evaluated.

It is essential to stress the fact that we are not here concerned with the accidental contamination of foods, nor with the deliberate adulteration of food, which was so common in years gone by until it was largely curbed by food laws. The misuse of chemicals for deceptive purposes or through ignorance or carelessness is, of course, illegal and outside the scope of any definition of a food additive.

It follows that a food additive should serve a useful purpose directly or indirectly benefiting either the individual consumer or the public at large.

FUNCTIONAL CLASSES OF FOOD ADDITIVES

From the functional standpoint intentional food additives may be grouped into four major categories:

1. Additives whose principal purpose is to enhance or restore nutritive value. These include vitamins, both natural and synthetic, mineral salts, and certain amino acids.

2. Additives whose principal purpose is to maintain qualities of freshness or to protect against deterioration. These include *a*) agents directed against chemically-induced or catalyzed changes, such as antioxidants, sequestrants, and enzyme inhibitors; *b*) agents directed against physically induced changes, such as emulsion stabilizers, anticaking agents and humectants; *c*) agents directed against microbially induced spoilage or hazard, viz. inhibitors of bacteria, yeast, or molds such as sulfites, benzoates, propionates, sorbates, and antibiotics.

3. Additives whose principal purpose is to improve or impart sensory qualities to food, i.e. flavor, aroma, color, texture, or appearance. These include a large array of natural and synthetic flavoring, sweetening, coloring, thickening, bodying, and gelling agents, waxes and other resinous glazes, and even carbon dioxide as used for carbonation. Many of the protective agents in category 2 serve to maintain natural esthetic qualities of foods by preventing discoloration, or off-flavor development.

4. Additives whose principal purpose is to facilitate processing or handling such as dough conditioners, lubricants (used in extruding, pelleting or other food handling machinery), antifoaming agents, solvents, emulsifiers, etc.

This classification of food additives is by no means complete and, of course, represents a considerable amount of overlapping in functional effect. No specific mention has been made of acids, alkalis and buffer salts, yet the regulation and stabilization of pH plays a vital role in many of these functions.

However, it should be apparent that the uses to which intentional food additives are put in modern technology do, in fact, result in real advantages to the consumer in terms of nutrition, sanitation, quality, convenience and economy, advantages which must be weighed against any possible hazards arising from their use.

Now let us consider some of the categories of so-called nonintentional or incidental food additives. These comprise substances used for some purpose in the raising or production of food which, despite good agricultural or technological practice, leave residues in the food:

1) Pesticides, including insecticides, fungicides, rodenticides, acaricides, nematocides.

2) Plant growth regulators including hormones, defoliants, herbicides, sprouting inhibitors.

3) Animal growth promoters including certain antibiotics, organic arsenicals, and estrogenic substances, and possibly also enzyme preparations.

4) Veterinary drugs including coccidiostats, antibiotics, tranquilizers, parasiticides.

5) Processing equipment including kettles, trays, piping, valves, conveyor belts, etc. of metallic, rubber, or plastic composition together with the finishes, lubricants or detergents which may remain on their surfaces.

6) Packaging materials including metals, plastics, paper, fabric, rubber, or wood, and the lacquers, coatings, plasticizers, pigments, and other components designed to impart certain physical properties adapted to the respective end uses of the containers or wrappers. Adhesives should also be included in this category.

There is no sound reason to believe that the proper use of pesticides and growth regulators impairs the nutritive value of food. On the contrary, by preventing rot, blight, and other plant diseases, by protecting against the depradations of insects, and by permitting harvesting under optimum conditions of yield and quality, agricultural chemicals contribute materially to the available food supply and to upgrading its nutritional quality and consumer acceptance. Prior proof of safety of residues of chemicals used for these purposes is, of course, required under the law. However, misuses are known to occur at times, resulting in the presence, for example, of DDT or penicillin in milk, or of aminotriazole in cranberries. Though such instances may constitute violations of legal tolerances they should not be construed as seriously jeopardizing the health or safety of consumers, if for no other reason than that the levels actually found are generally well under maximum safe levels, and these, in turn, are based on large margins of safety applied to 'no-effect' dosages for animals. Transgressions of tolerance limitations call for regulatory action but not for public alarm.

As was anticipated, the migration of additives from processing equipment, containers or other packaging material has posed many difficult problems under the

new law designed to insure the safety of food additives. Of the large number of substances used in the fabrication of such products, many either do not migrate at all under normal conditions or, if so, in such small quantities as to present analytical difficulties which, in some cases, defy solution. Examples are the identification and determination of traces of petroleum hydrocarbons, or of low molecular weight polymers from wax paper and plastic film. To meet this situation the Food and Drug Administration has indicated its intention either to waive the statutory requirement of a method for determining the amount of an additive in food, or to regard as 'zero' any amount not detectable by a sufficiently sensitive analytical method.

That vigilance is required in respect to chemicals used under conditions where they may become unintentional additives to food is indicated by the recent discoveries that hyperkeratosis (X-disease) in cattle was due to a toxic lubricating oil used in a pelleting operation. Though it may not have involved hazard to human health, this episode revealed an unanticipated source of toxicity and justifies attention to the safety of all substances foreign to foods regardless of whether their presence is intentional or unintentional.

This condensed summary of the functions of food additives provides ample evidence of their technological justification. It is worth repeating that no use of a food additive is permissible which is deceptive or which conceals inferiority. It remains to discuss whether food processing generally, and food additives particularly, affect the safety of foods and if so, how this is established.

SAFETY OF FOOD ADDITIVES

Since the enactment of the Food Additives Amendment, it has become apparent through orders of the Food and Drug Administration that hundreds of food additives are deemed safe by appropriately qualified experts judging either by experience in use, toxicological tests, or both. Many more have been considered safe and approved for use by the Food and Drug Administration or the Meat and Poultry Products Inspection Divisions of the Department of Agriculture. In addition a large number of additives have been granted extensions for 1 year beyond the date when the amendment became fully effective, from which it may be inferred that the use of these substances involves no undue risk to public health. The final status of many additives will depend not so much on toxicity data as on evidence establishing whether or not they may become components of food in quantities sufficient to require consideration from the standpoint of safety or, if so, whether specifications for identity and purity are adequate to insure that the substances will be of satisfactory food grade.

It is not the purpose of this presentation to delve into presently unresolved questions concerning the safety of individual food additives. However, mention may be made of some of the broader problems that have beset toxicologists in industry and regulatory agencies. Several years ago inquiry was made into the matter of emulsifying and wetting agents to determine whether surface activity per se was a sign of potential toxicity. As a result a report was issued by the Food Protection Committee in which it was concluded that there was no basis to support such a generalization and that each surface active substance would have to be evaluated independently (11). Some nonionic emulsifiers, like the polyoxyethylene esters of fatty acids, actually lose their surfactant effectiveness upon hydrolysis in the gastrointestinal tract. Another question dealt with the possible harmful or irritant effect of laurates. A study of this problem by an ad hoc expert committee has led to a recent report exonerating laurates as such (1).

Undetermined or conflicting views regarding several other basic questions of toxicology might well be referred to expert committees for guidance in establishing general policy. For example, what level of nondigestible oil, such as mineral oil or hydrocarbon-like oils migrating from wax-coated or from plastic wrappers, can be safely tolerated? Or what are the toxicological properties of certain classes of organic compounds and to what extent are they influenced by characteristic groups, e.g., sulfhydryl, allyl, vinyl, and cyclohexyl compounds? Or, can safe levels of certain elements like cadmium, mercury, chromium, fluorine, selenium, or arsenic be established, bearing in mind that some have nutritional value and yet may be carcinogenic under certain conditions? Is it appropriate to regard oxalate deposition in the bladder, or any lesion which under exaggerated experimental conditions may ultimately develop tumors, as 'precancerous' even when they are self-limiting or reversible upon removal of the toxic insult? Is synergism a sufficiently real, rather than theoretical, possibility at the level of concentration of food additives to demand consideration generally or only in special cases? These are some of the vexatious problems that will have to be dealt with in the coming years as experience with the new law unfolds.

It is pertinent to recall the statement of the Joint FAO/WHO Expert Committee on Food Additives (6) to the effect that while it is impossible to establish absolute proof of the safety for use of any additive for all human beings under all conditions, critically designed animal tests can provide a reasonable basis for evaluating safety at a specified level of intake which should be "substantially below any level which could be harmful to consumers."

According to the Food Protection Committee of the National Academy of Sciences-National Research Council, safety is the practical certainty that injury will not result from the use of a substance in a proposed quantity and manner (9). The interpretative regulations under the Food Additives Amendment define safety more affirmatively to signify that "there is convincing evidence which establishes with reasonable certainty that no harm will result from the intended use of the food additive" (4).

Apparently then, the conditions of use are generally agreed to be all-important. But what about the nature

or degree of harm that would constitute hazard? If a food process or a food additive should so damage a food as to render it unpalatable or inedible, does it follow that it is unsafe? Foods that are not eaten make no contribution to nutrition; on the other hand, unless they are eaten they are not unsafe. As a matter of fact the proper use of additives to protect food against deterioration in flavor, odor, or appearance, render it safer than they would otherwise be. A change in concentration of a particular nutritional component as a result of processing does not justify the inference that harm will result from the ingestion of the food. If that were so, the milling of wheat to remove the bran and germ, or pasteurization of milk with concomitant loss of ascorbic acid, would be considered injurious. In evaluating the safety of a food a distinction must therefore be drawn between any effect on its nutrient composition, which may occur before the food is consumed, and an injurious effect which may occur after the food is eaten, i.e. as a result of local action in the gastrointestinal tract or systemic action following absorption. Excessive exposure to heat, light, air, and water may reduce nutritional values. Traces of copper may reduce ascorbic acid content, sulfur dioxide may destroy thiamine, mineral oil may inhibit absorption of oil-soluble vitamins, but in none of these cases can we say that the effects are toxic. If the physical conditions of treatment or the use of chemicals are such as to cause loss of nutrients in a food that is a significant source of the particular nutrients, it might be proper to regard them as unsuitable or unacceptable, but not on the ground that the food is unsafe. This is not to say that effects of processing on vitamin retention, on the digestibility and utilization of proteins and fats, and so forth, are not appropriate matters for evaluation by food scientists and for control by regulatory agencies, but the distinction between nutritional and toxic effects should be kept clearly in mind.

This suggests another problem that has arisen in experiments for the evaluation of safety. So strongly has the urge been to establish a no-effect level in animals at 100 times the human use level (as will be discussed later), that experimental rations have often been devised in which the test substance displaces a significant portion of the dietary essentials. It is not always possible for animals to compensate by eating excessive quantities of the diet mixture. Hence, the recommendation of the FAO/WHO Expert Committee that "dietary levels higher than 10 per cent should not be used" ought to be consistently adhered to. In any case safety appraisals should clearly differentiate between organic or functional effects induced directly by the test substance on the animal organism and those resulting from inadequate or irregular food intake or from changes in specific nutrients prior to ingestion.

The question may reasonably be raised at this point: 'What have the procedures for safety evaluation to do with the impact of food technology on safety?' The answer depends, of course, on what we mean by safety. It may not be responsive but it nevertheless is accurate to put the statement in reverse, that is to say that the time, cost, and uncertainties of establishing safety have an increasingly important bearing on progress in food technology which the food additives law is designed to foster.

Decisions as to safety usually rest upon animal experiments which, even when well designed, entail more or less arbitrary conditions. The selection of dietary feeding levels is, of course, critical and several important facets of this phase of safety appraisal warrant discussion. For want of a more rational basis upon which to transpose safe levels for animals to safe levels for man 'under the conditions of intended use,' the safety factor 100 is generally employed with the blessing of various official and quasi-official agencies. However, the factor is not always applied to the same parameters. Professor Frazer said in 1953 (7) that "It has been agreed on a more or less arbitrary basis that food additives should be tested at various dosage levels *up to* 100 times the standard dietary dose" (underscoring supplied). He pointed out however, that it was not clear how this should be applied. To quote further, "some authorities demand that no effects should result from the hundred-fold test dose, while others have not accepted substances that conform even to this rigorous criterion, and still others have suggested that substances should be rejected on the basis of invisible and undemonstrable changes that may have occurred. This last view denies the whole basis of observational and experimental science, and clearly makes any form of biological testing otiose." In view of the practical impossibility of establishing a no-effect dose with any degree of precision, Frazer proposed that if significant modification of structure or function could be observed in chronic feeding tests in less than half the animals at a 100-fold dose level and no effects were observed at 10-fold, the proposed dietary level could be regarded as acceptable. Nevertheless the FAO/WHO Expert Committee (10) (of which Professor Frazer was a member) stated that "where the maximum ineffective dose in animals is calculated in g/kg body weight a margin of safety of the order of 100 . . . is adequate" in the absence of any evidence to the contrary. This principle has been adopted by the U. S. Food and Drug Administration with the added explanation that "a food additive for use by man will not be granted a tolerance that will exceed $\frac{1}{100}$ of the maximum amount demonstrated to be without harm to experimental animals" (5). To justify the 100-fold safety factor on the ground that man is 10 times as sensitive as other species to certain drugs and poisons, and that individuals in various states of health and disease may vary 10-fold in sensitivity, is pure rationalization. Suitable comparative data on relative species sensitivity are meager at best and are predicated largely on acute or subacute toxicity. The relationship could be entirely different under conditions of chronic or life-time dosage.

At any rate the phraseology of the FDA regulation quoted above is ambiguous and confusing for several reasons: *1*) because it does not specify the units in which the 'maximum amount' is expressed, i.e. whether as ppm

of test diet or mg per kg body weight of animal; *2*) because it does not make clear that the basis of expression should be the same under both the test and proposed use conditions; *3*) because in the legal sense a 'tolerance' is the allowable level in a particular food, rather than in the diet as a whole, and is set not at the maximum safe level but at a level no higher than necessary to achieve the desired physical or other technical effect; and finally, because it fails to specify that calculation of the human dietary levels should be on a dry weight basis similar to that employed in the preparation of test diets. Failure to take these factors into account could result in many-fold exaggeration of the assumed 100-fold safety factor for the reasons, that laboratory animals consume several times as much food as man in terms of dry weight (or calories) per unit of body weight; foods in which tolerances are established generally constitute only a fraction of the total dietary intake; and human diets are composed of foods that collectively consist of approximately two-thirds water.

In estimating probable levels of human intake, it has become customary to regard the maximum ingestion level of a particular food or class of foods as typical of actual daily intake. Moreover the assumption is made that such ingestion is continuous throughout a lifetime. These exaggerations, in effect, further multiply the real magnitude of any assumed safety factor applied to dosages. It would seem logical therefore in interpreting the results of animal studies that more consideration be given to 'patterns of consumption' especially as related to foods consumed only occasionally, seasonally, or for limited periods during the life cycle. Perhaps dietary levels should be estimated on a weekly, monthly or annual basis, rather than daily, and test feeding conditions adjusted accordingly.

The concept of zero tolerances for certain additives, especially those found (or suspected) to induce cancer in animals, raises questions of the sensitivity of analytical procedures and what will happen when future methods will be devised of greater sensitivity than those currently in use. The principle should be recognized that at some finite minimal dose any substance can be administered with reasonable certainty that no harm will result. Hence insistence on 'absolute zero' can only lead to absurdity. For instance a food ingredient containing a pesticide residue within a permitted tolerance might be said to adulterate foods in which standards of identity do not provide for such residues; pure chemicals containing traces of arsenic or 'heavy metals' within acceptable tolerance limits might be said to adulterate foods if any of these trace elements should be barred as carcinogenic to animals.

The consequences of a rigid, nondiscriminatory enforcement of the philosophy expressed in the Delaney Clause are not to difficult to imagine. The observation in feeding trials that a chemical component, naturally presents in spices or essential oils, induced cancer could eliminate that substance from foods regardless of whether it was present in its natural state or produced synthetically. It has been shown that roasted coffee (8) and smoked meat and fish (2) contain demonstrable amounts of benzopyrene and possibly other carcinogens. The finger of suspicion has been pointed to polymerized or epoxidized fats. Green leafy vegetables, alfalfa and other grasses as well as several other common foods have been found to contain measurable quantities of estrogens, another class of potential carcinogens. Compounds containing the allyl group have been found to vary considerably in toxicity, some being hepatotoxic or even tumorigenic upon chronic ingestion; nevertheless this class of compounds is responsible for the characteristic taste of onions, mustard, radish and a host of other common vegetables and seasonings.

If experience can not be relied upon to indicate that the ancient and honorable practices of preservation by smoking, frying in deep fat, and seasoning with common spices are safe within the meaning of the Delaney Clause, our food habits will have to undergo radical change. If on the other hand, the rule of reason prevails and it is recognized that there are safe levels even of dietary carcinogens, revocation of the absolute legal prohibition will follow.

It would be helpful if standards could be adopted to assess the weight of evidence advanced in support of causal relationships between dosage and response. For example, one might classify such relationships as ranging from negative to positive through intermediate steps, e.g. *a*) negative—unequivocal or proven beyond reasonable doubt; *b*) hypothetical—based on an assumption without proof, i.e. a tentative theory to explain certain effects and to serve as a guide for further investigation; *c*) suggestive—based on indirect or qualified evidence, accepted with reservation subject to further investigation or consideration; *d*) presumptive—based on sufficient evidence to justify a reasonable inference of a fact but not definitely proven; *e*) positive—unequivocal or proven beyond reasonable doubt. These gradations of interpretation might be considered in establishing whether or not a substance is 'found' to induce cancer in man or animals. It would seem reasonable that only presumptive or positive evidence be considered as sufficient ground for denial of a tolerance.

Emphasis has been placed thus far on the interpretive, rather than procedural, aspects of safety evaluation because it is in this area that generally acceptable principles have yet to be established. Some eminent toxicologists have directed criticism toward the frequent insistence on chronic feeding studies as a sine qua non in the assessment of safety. Shorter and more economical procedures employing biochemical, metabolic or radiotracer techniques have provided sufficient information in some instances, but these have been relatively few compared to long-term studies. Where the substances under test are closely related to common components of foods, as for example mono- and diglycerides, sorbic acid, simple fatty acid esters, etc., short term feeding tests or metabolic studies may be adequate.

Toxicological feeding studies must be planned and

executed with due regard for possible metabolic pathways and for biochemical, pharmacological, and morphological effects. In the investigation of sequestrants, for example, attention must be directed toward all phases of trace metal absorption and utilization, particularly hematopoiesis and systems involving metalloenzymes; studies of neurotoxic insecticides require consideration of cholinesterase activity; surface active substances may influence intestinal absorption and, if absorbed intact, the permeability of various cell membranes; compounds structurally related to vitamins or hormones may have antimetabolic effects; and so on.

The use of tissue culture preparations or of specific cell suspensions show considerable promise of yielding fundamental toxicological data but these, like functional tests of target organs, must be interpreted with caution in relation to potential chronic effects in intact animals. The same may be said of single metabolic systems like the rate of glucose utilization, or of neurophysiological reactions like the conditioned reflex, as indicia of toxicity. Useful as they may be for purposes of revealing mechanisms of action, it is impossible to forecast when, if ever, these procedures will supplant long-range feeding trials in different species of animals as criteria for safety evaluation of food additives.

CONCLUSION

We have entered upon a new era comparable to that following Pasteur's foundation of the science of bacteriology which has had such a profound effect on the course of food technology. No single discovery but rather the general growth of chemical science and industry in our own time has had an impact on food production and processing, as on every other aspect of our daily lives, which demands careful attention to potential risks as the price of enjoying benefits.

Fundamentally the problems of safety are not new. In olden days they were attacked by the method of trial and error and the criteria were simply manifest injury or death. Food patterns were established by the process of elimination. Today science has not only given us many new and useful substances but has taught us to identify the chemical components of our foods. What is more significant however is the fact that we now use techniques and tools capable of revealing slight or subtle physiological changes induced by chronic or lifetime ingestion.

The application of these advanced methods and of higher standards of safety evaluation, proceeding concurrently with progress in agriculture, nutrition, and food technology, has added a new dimension to the wholesomeness of our food supply. It is to be hoped that as new legislative and regulatory controls become adapted to changing conditions, balanced judgement will prevail so that technological progress can be assured at no sacrifice to the quality, variety, and safety of our food.

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Nutritional aspects of pesticides and the use of agricultural chemicals

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AMONG THE CAUSES impeding the development of agricultural production, losses of food due to the depredations of pests and diseases are not unimportant.

FOOD LOSSES FROM PESTS AND DISEASES AND PROBLEMS ARISING ABOUT THE USE OF PESTICIDES

Referring to a report of the U.S.D.A. Agricultural Research Service (1), losses to crops during production due to insects and diseases, mechanical damage, hail, weeds and inadequate harvesting amount to about $8.3 billion annually in value of products lost. These losses constitute 20% of the potential production of all farms and forest growth. They are estimated as following:

Diseases of crops	6.9%
Insects attacking crops	4.7%
Mechanical damage, hail and weeds	5.9%
Harvesting losses	2.7%

Furthermore, losses during storage, marketing and processing account for $3.9 billion, which is equivalent to about 14% of the total value of the products or to over 6% of the total farm and forest production.

McDonald (41) relates that in the Canadian province of Saskatchewan, during the 5-year period 1951–1955, the 4 principal insect pests—sawfly, wireworms, cutworms and grasshoppers—were responsible for destroying 148 million bushels of wheat, 25 million bushels of oats, 15 million bushels of barley and some rye and flax. In Great Britain, over the 12-year period 1946–57, over 7% of sugar beet crop was lost by attacks of virus yellow. Over a 10-year period, the equivalent of 3,000 hectares of Brussels sprouts, 14% of the total production, was lost annually through aphid damage. Over a 12-year period, losses of oats from fruit fly attack amounted to the equivalent of 100,000 hectares annually, or 14% of the total acreage (23). In Germany, according to Steiner (52), damages to grain due only to grain weevil amount annually to 300,000 tons. In the Mediterranean countries, the olive fly destroys annually about 20% of the crop (3).

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Many serious pests and diseases can be kept under control by methods of good husbandry, including proper crop rotation, the use of sound, approved seed and the development and planting of resistant crop varieties. There have also been limited successes in the field of biological control, i.e. the introduction of a parasite, bacterium or virus to destroy the larvae of a dangerous insect pest. However, the use of pesticides has outweighed every other method of control, for it appeared in most cases as more efficient and more applicable on a large scale.

Difficulties however have been encountered: the adaptation of several species of insects to various pesticides results in the rising of resistant strains (36, 11), the destroying of certain species leads in some cases to a rupture of the biological balance (36), furthermore, the use of toxic compounds for the control of insects and diseases appears not always to be safe for mammalia.

The problem raised by the presence of pesticide residues in food has aroused considerable anxiety both among public health authorities and certain individuals. At present, there is no evidence of effect from long term ingestion of the residues of pesticides on man. However, as outlined recently by Zavon (61), until our methods of clinical evaluation become far more refined, we will probably be unable to detect effects from the residues found at present.

Nevertheless, this statement must not allow us to draw our attention away from the problem. As pointed out by Barnes (6), reassurance, if it can continue to be given, needs to be based on whatever scientific data can be assembled. This must show in the first place the actual quantities of the pesticides which are being consumed and then the relation that such a dose level may have to those doses known to produce the first signs of poisoning. At the present time, the problem is far from being solved; for, if systematic checks of the foodstuffs presented on the markets allow a rather accurate estimation of the situation in some countries, one is completely ignorant of the situation of most of the others. Furthermore, it becomes complicated by the fact that the importance of pest control and the food balance sheets largely differ from one country to the other.

Among the foodstuffs that may contain residues of pesticides, fruits and vegetables have been subject to the largest number of studies and measures of regulation. The consumption of fruits and vegetables however increases with the standard of life of the populations and there are many countries where it amounts to very little.

On the other hand and in spite of the request of the WHO experts (60), too little attention seems to be drawn to the possible contamination of 'basic foodstuffs' which are continually consumed, such as cereals, flours and products derived therefrom, milk, meat and fats.

AMOUNTS OF RESIDUES THAT APPEAR IN FOODS UNDER CONDITIONS OF USAGE

Fruits and vegetables. In the United States and Canada, limits of tolerances for pesticide residues in raw agricultural commodities have been established by law and the sample testing of foodstuffs sent to market is carried out systematically. This procedure makes known the amounts of residues present in fruits and vegetables and insures that the level considered as 'safe' is not exceeded.

These limits of tolerances are not yet in force in Europe for various reasons which have recently been explained (18). Nevertheless, some European countries have already taken steps to control the amount of pesticide residues that may remain in or on food, either through the establishment of a minimum interval between final application and harvest, with or without tolerances, or by means of restrictions on the sale or on the use of highly toxic pesticides.

In the European countries where quality-control of markets is performed, it appears that pesticide residues in fruits and vegetables seldom reach the limits of tolerances admitted in the United States and Canada and generally are distinctly below. Here are some examples.

Raucourt *et al.* (45–50) showed after several years of experiments in France that arsenical treatments correctly performed up to 30 days before harvesting leave deposits of arsenic on apples that are within the requirements of the American tolerances (2.3 ppm of arsenic in U. S., 2 ppm in Canada).

Hardon (34) established by means of controls made in Holland in 1954 and 1955 that in a total of 633 samples of fruits and vegetables collected on the markets, only 2, i.e. 0.3%, retained parathion residues in amounts higher than 1 ppm (limit of tolerance admitted in U. S. and Canada). Furthermore, 406 samples, i.e. 64%, were completely free of parathion residues. Similar results were obtained by Hardon (34) in 1957: in a total of 881 samples of fruits and vegetables, none of them presented residues of parathion in amounts higher than 1 ppm and 739 samples, i.e. 84%, were free of parathion.

In Belgium, many samples of apples and pears coming from industrial orchards were analysed during the winter 1958–1959 (19); these fruits had received from 4 to 12 treatments with parathion and had been kept in refrigerators during 44 to 99 days. Residues at the time of consumption ranged between 0.01 and 0.06 ppm, the total amount of residues being localized in the peel only.

In Italy, residues of parathion in olives which were very high some years ago, are now, as a result of an improvement in the techniques of pest control, substantially reduced (5).

A control of DDT residues on apples and various vegetables has been done in Holland (34). The residues appear variable from one year to the other probably as a result of the variability of the climatic conditions; in 1955 and 1956, however, they were extremely low.

Controls performed in Switzerland by Eichenberger (22) showed that residues of DDT on swiss cherries were not high; 71% of the samples were free of residues and 4% had residues ranging from 2.6 to 7 ppm; in none of the cases was the limit of tolerance of 7 ppm established in North America exceeded. On the other hand, cherries imported from Germany had higher amounts of DDT residues; 12% of the samples had more than 7 ppm DDT and 23% only were free from residues.

In general and as far as results of analyses are available, the situation of fruits and vegetables from the point of view of the pesticide residues appears to be favourable in the countries where measures are applied for avoiding hazards to the consumer.

It is also worth while to remember that the results of analyses are generally related to whole and nonwashed fruits and vegetables and that peeling, scraping and washing, as usually made by the consumer, are able to remove partially and sometimes totally the residues.

One must however not ignore that there are some countries where the population is less hygiene conscious and where, among others, the presence of certain parasites, such as the fruit flies, may lead the user to apply the pesticides at the time of harvesting or a few days before.

Truhaut and Viel (55) pointed out some cases of intoxication which seem to stem from this kind of misuse. Such situations justify the recommendations made by the experts of FAO (25) and WHO (60).

As to the effect of the pesticides on the quality of the fruits and vegetables, it is established that BHC, lindane and chlordane impair the taste, particularly in the case of tubers and roots grown in soils treated with these insecticides. Furthermore, a slight decrease of the acid and sugar content has been observed in oranges and apples treated with arsenicals (8).

Tubers and root. Among the compounds used for the treatment of soils, DDT, lindane, aldrin, dieldrin, chlordane, toxaphene and heptachlor accumulate and may persist several years. According to Gunther (32), there has been accumulating evidence that some soil insecticides are absorbed by certain root crops. However, the evidence has not been presented that this situation is greatly different from ordinary topical application, that is, that the insecticide actually contacting the growing root or tuber is merely penetrating into the tissue making contact.

Residues of various chlorinated hydrocarbons, of parathion and EPN have been found in amounts generally lower than 1 ppm in potatoes, carrots, tomatoes

and cucumbers grown in soils treated 1 year before with these insecticides (32).

Residues of aldrin or dieldrin in amounts not exceeding 0.9 ppm were found on the one hand by Steiner (53) and on the other hand by Dormal *et al.* (19) in carrots grown in soils treated with different formulations of aldrin a few days before sowing. Steiner, however, demonstrated that these residues were localized in the skin only and were easily removed by scraping.

Cereals and flour. Cereals, flour and stored food often are attacked by insects and mites. The principal means of control are fumigants and contact poisons. Among the fumigants, methyl bromide, ethylene dibromide, ethylene dichloride, ethylene oxide, hydrocyanic acid and phosphine are the most commonly used.

During the fumigation, a considerable amount of gas is taken up by the foodstuff by physical adsorption. This is followed by a desorption during the airing period following the treatment. The desorption is never total. Residues of the fumigants are still found in the foodstuffs after ventilation, but in very small amounts which are estimated as free of hazards for man and mammals. However, with some of these compounds part of the physically adsorbed gas may react chemically with the foodstuffs.

Residues of methyl bromide in wheat have been experimentally studied by Winteringham, Bridges *et al.* (12, 58, 9, 57). It appears that methylation of proteins accounts for most of the reaction; nevertheless, during a normal fumigation, the reduction of methionine and histidine content does not exceed 2 and 1.3%, respectively. According to Winteringham (57), such levels of destruction are probably small compared with the natural variations from one wheat crop to another. The possibility of destruction of vitamine B has been investigated by Clegg and Lewis (17); no evidence of loss was found under the conditions of methyl bromide fumigation.

Residues of ethylene dibromide in wheat have been studied by Bridges (10). In spite of the high physical sorption of the fumigant and its slow rate of airing, the amount of chemical reaction between it and the wheat is small at room temperature. No significant changes are likely in the nutritive value of wheat as a result of ethylene dibromide fumigation. A tolerance of 50 ppm of inorganic bromide residues (calculated as Br) has been set by the F.D.A. Ethylene oxide may destroy partially thiamine (2).

An international group of WHO experts on toxic hazards of pesticides to man emphasized that whenever fumigants are used for the control of infestation in stored products, less residue hazard is created (60).

A more important problem arises with the contact poisons such as DDT and lindane which are mixed with the grain or applied to the sides of the containers. Although there may be some volatilization under conditions of good ventilation (16) and during storage (62, 40) most of these compounds are absorbed by the food and only cleaning processes (washing, cleaning, sieving, rubbing, etc . . .) are able to remove them.

Considering the cumulative effects of these insecticides and the fact that cereals may constitute a large part of the human and animal diet, the treatment of stored foodstuffs with DDT and lindane is not authorized in some countries among which are Algeria, Belgium, Canada, Netherlands, Switzerland and the United States. On the other hand, Great Britain and its overseas territories, France, Italy, and Denmark allow it with established limits of tolerances for the residues.

Parkin (44) has reviewed, with much objectivity, the advantages and disadvantages of this technique and points out that if the use of DDT and lindane is not always free from hazards for the consumer, it may be a necessity in the countries where insect infestation during storage can lead to serious crop failure or even to famine.

Allethrin, pyrethrins and piperonyl butoxide also are used for the treatment of stored food. During storage, these compounds penetrate into the foodstuffs but do not present the hazards of cumulative effect. Zeumer and Neuhaus (63) have investigated the behaviour of piperonyl butoxide in rye before and after grinding. In the United States, the limits of tolerances are fixed as following: 2 ppm for allethrin, 3 ppm for pyrethrins and 20 ppm for piperonyl butoxide.

Vegetable oils. Most of the chlorinated hydrocarbon insecticides and phosphoric esters easily penetrate into the oleaginous constituents of the plants and significant residues may remain during months in the oils.

The control of the olive fly (*Dacus oleae*) is an important problem for the Mediterranean countries.

According to the results of Alessandrini (3), the residues of all chlorinated hydrocarbons experimentally tested on olive trees were very high in olive oils, indicating that the use of such compounds is definitely not to be recommended in olive trees.

During the years 1954 and 1955, the residues of parathion in olive oils were also high; they ranged from 0.2 to 36.8 ppm.

Different techniques were applied the following years with the object of reducing these residues. According to the latest report presented by Alessandrini *et al.* (5), it appears that the situation has improved from one year to the next and that at the present time, there is little likelihood of finding olive oils heavily charged with phosphoric esters. The percentage of samples free from parathion which was of 6.9 in 1955 reached 61.8 in 1958. Furthermore, 76% of the samples analysed in 1959 had a content of parathion lower than 1 ppm (limit of tolerance set in U. S. and Italy). For diazinon, the samples free from residues, which was of 5.4% in 1955, reached 40% in 1958.

Rogor (dimethoate) was also investigated, it presents the advantage not leaving any residue in the oil (5).

Referring to Alessandrini (3), the refining of olive oils by the usual techniques allows only a partial elimination of the parathion residues.

The quality of vegetable oils may also be impaired by the use of some insecticides. It has been shown by Gilpin *et al.* (31) that peanuts are readily contaminated with

the flavour and odour of BHC and lindane. Refined peanut oil originating from soils treated with various organo-chlorine compounds was used in french frying of potatoes. The oil from BHC plots had a musty odour and imparted a musty odour to the fried potatoes. Oil from aldrin plots had a slightly 'strong' odour at 180°C. In general, the oils from the several treatments appeared less stable and smoked more at 180°C than oil from control lots (8).

Milk, meat and fat. The residues of most organo-chlorine compounds on forage crops used as animal feed, and on the animals themselves, as a result of spray application, accumulate as such or are metabolized in various tissues of the cattle and are excreted in milk. DDT was found to be present in the heart, liver, spleen, kidneys, leaf and caul fat and in edible cuts such as T-bone steak, rump cut and lean meat from the neck of beef cattle fed hay containing DDT residues of approximatively 180 ppm for a period of about 5 months. In all cases, the greatest amount was found in the fat (14).

While all the organo-chlorine insecticides share the property of being stored in the body fat, the dynamics of this storage differ from one compound to the next. Lehman (39) has clearly brought out the differences and demonstrated that the storage pattern of aldrin, dieldrin and endrin approximate that of DDT, while lindane and methoxychlor follow a different pattern.

Dieldrin, fed in the diet of dairy cows at levels as low as 0.1 ppm appeared in the milk at the end of 6–12 weeks; tissue analyses at the end of the feeding period indicated that dieldrin was present at relatively high concentrations in fats and that the amounts found in other tissues were proportionate to their fat content (28–30).

Considering that milk, meat and fats, as well as cereals, form a staple food for many communities, special attention should be paid to the possible contamination of these foodstuffs. Though, at present, there is no evidence of effect from long term ingestion of the residues of pesticides in milk and meat, their presence in milk is not allowed in many countries; in others, among which is Belgium, the treatment of cattle, and stored foodstuffs with organo-chlorine insecticides (with the exception of methoxychlor) is forbidden.

Hayes *et al.* (35) have experimentally established that people abstaining from meat deposited in their fat only about half the concentration of DDT (2.3 ppm) as did people in the general population (4.9 ppm) and that meatless meals served in a cafeteria catering for meat abstainers contain only about a quarter as much DDT and DDE as did meals served in ordinary restaurants. The authors believe that there is strong evidence that essentially all storage of DDT and DDE in people without occupational exposure results from the presence of these compounds in the diet, especially, but not exclusively, in fats of animal origin.

DDT has been found by Laug *et al.* (37) in human milk. The records of all cases examined indicated that none of the subjects were occupationally employed as pesticide operators and that the greatest possible amount of DDT to which they were exposed must have come from the diet.

FATE OF RESIDUES AFTER PROCESSING OF FOODSTUFFS

Cleaning, washing and peeling. Washing of the fruits and vegetables permits the removal more or less completely of the residues when they have not penetrated into the plant epidermis (33). The speed and rate of penetration of the residues into the plant tissues depend essentially upon the nature of the compound, the type of formulation and the kind of plant epidermis. Generally, the residues of compounds in the form of emulsifiable concentrates penetrate with surprising rapidity into the cuticular tissues. Phosphoric esters in wettable powder form seem to penetrate equally rapidly. Powders, on the other hand, have a much lower power of penetration (32).

It has been observed during experimental treatment that the residues of parathion, chlorthion and isochlorthion left by emulsion treatment almost completely penetrate cherries in less than 3 hours after spraying and were unable to be removed by washing (20). On the other hand, residues of thiram left by wettable powdering on strawberries were eliminated at the rate of 60–100% after 1 minute steeping in water (19). For apples, pears, peaches and citrus fruits, most of the residues only penetrate into the peel and are completely removed by peeling (19, 7, 13). Exception must be made for the compounds with a systemic effect, which circulate throughout the plant. Scraping of carrots grown in aldrin-treated soil removes the residues (53).

Ordinary cleaning processes prior to milling remove a large proportion of DDT and lindane dusted on grain during storage (40, 44, 64, 62, 56) and a large part of what remains is lost with the bran during milling (44, 62).

Processing of fruits and vegetables. Taschenberg and Avens (54) have demonstrated that juice made according to commercial procedure from grapes containing residues of 7–10 ppm DDT was found to be free from DDT, while relatively large amounts was present in the pomace. Similar results were observed by Frear and Cox (26). No DDT was found in jam samples made from field-treated fruits whether or not DDT was present in the finished pulp.

In the processing of DDT- and DDD-treated tomatoes into juice, Miller *et al.* (42) showed that commercial washing removed approximately 80% of the residues irrespective of the rate of application. The remaining 20% of residue was concentrated in the waste, more especially on the skins, and only traces of residues (0.02–0.13 ppm) were found in the juice.

Dormal *et al.* (21) studied the rate of elimination of phosdrin, dipterex, parathion, isochlorthion and DDT residues on treated spinach, peas and beans through blanching and commercial canning processes. Blanching results in a notable reduction of phosdrin and dipterex residues, elimination of 40–85% of parathion and isochlorthion residues and 20–40% of DDT residues. Commercial sterilizing results in a complete elimination of phosdrin and dipterex residues, elimination of 70–95%

of parathion and isochlorthion residues and 40–60% of DDT residues.

Analyses of several organic insecticides residues in peeled, canned, treated tomatoes were performed by Alessandrini *et al.* (4). On 36 samples, 31 showed no residues. In 2 samples treated with lindane, residues amounted to 0.12 and 0.13 ppm. In the 3 samples treated with dieldrin, the amount of residue was slightly higher, i.e. 0.2, 0.4 and 0.6 ppm. Similar results were obtained by Miskus *et al.* (43) and Erwin *et al.* (24). Other examples of losses of organic insecticides from processing of several treated food products were published by Gunther (33).

Residues of inorganic compounds are generally more resistant. Robinson *et al.* (51) observed in a series of tests with tomatoes sprayed with copper containing fungicides that, although commercial washing processes removed about 50% of the residue, peeled tomatoes and juice showed a significant increase in copper content over the fruit and juice from the control lots. The copper content of the canned juice decreased during storage, as it plates out on the inside of the can. Frear *et al.* (27) found that in the processing of lead arsenate-treated grapes into juice, 17% of the lead and 35% of the arsenic went into the pressed juice.

In an experiment on commercial sterilization (washing and heating at 118°C) of celery treated with organic tin and organic arsenic containing fungicides, Dormal *et al.* (19) showed that 92–98% of organic tin and 25–50% of total arsenic were removed.

Cooking and baking. In experiments conducted by Carter *et al.* (15), no loss of DDT content was recorded on beef meat after roasting, broiling, pressure cooking, braising or frying.

Winteringham *et al.* (59) found a concentration of 14.6 ppm bromo-DDT in flour milled from grain carrying 40.2 ppm of this compound and 11.2 ppm in bread made from the flour. Some of the grain and bread were fed to hens and rats; the insecticide was found in all the tissues examined. Bromo-DDA was identified in urine of man following ingestion of some of the bread.

On the other hand, Leggieri (38) showed that the DDT content of bread was about ½–⅓ that of the flour and Zeumer and Neuhaus (62), baking flour containing 6.8 ppm DDT or 1.2 ppm lindane, obtained bread with less than 0.2 ppm of either insecticide.

CONCLUSIONS

The use of pesticides in agriculture appears to be a necessity. As pointed out by Barnes (6), the effective control of toxic hazards from residues is the concern of the public health authorities in individual countries. It is clear that problems take different forms in different countries. Where food is abundant and public health standards are high, the authorities may be justified in insisting on the very minimum of contamination of food by pesticides. In poorer countries with limited sources or stocks of food the preservation of this food may be more important than the hypothetical hazard from the pesticides in food.

The appraisal of the hazards to the consumer first of all requires an exact knowledge of the amount of the various pesticides present in food and of the proportion of such treated food in the diet.

At present, we have a rather good knowledge on the amounts of pesticide residues present in fruits and vegetables under conditions of usage. However, too little attention has been payed regarding residues in other food, such as cereals, milk and meats, which may constitute staple foods for some communities.

Cleaning and processing of treated foods appears in most cases to reduce the amount of residue to a small level, sometimes to remove it totally.

In spite of lack of positive evidence of harm to the national health in communities where food treated with pesticides is consumed, efforts toward the development and the use of nonpersisting pesticides merits encouragement.

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Einfluss der Verarbeitung in Industrie und Haushalt auf den Nährwert von Fleisch, Getreideprodukten und Hülsenfrüchten

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BEI BE- UND VERARBEITUNG ist in den letzten Jahren neben den Fragen der Haltbarmachung und Schmackhaftigkeit der Nahrung das Problem der Nährwerterhaltung oder gar Nährwertverbesserung mehr und mehr in den Vordergrund getreten (18. 19. 20. 46. 73). Doch ist dieses Problem viel zu umfangreich, um im Rahmen dieses kurzen Beitrages auch nur annähernd erschöpfend behandelt werden zu können. Vor fast 10 Jahren hat Zimmermann (106) die Wirkung der Hitzebehandlung auf den Nährwert des Eiweißes in Nahrungs- und Futtermitteln ganz ausführlich dargestellt und ist, unter Berücksichtigung der bis dahin vorliegenden Literatur, ausführlich auf den Einfluß der Be- und Verarbeitung von Fleisch, Cerealien und Leguminosen auf ihren Nährwert eingegangen. Doch auch über neuere Ergebnisse liegen zusammenfassende Darstellungen vor, auf die im Text verwiesen wird. Daneben aber sollen Teilfragen anhand besonders charakteristischer Beispiele aus der neuen Literatur behandelt werden.

Zunächst sei kurz dargelegt, auf Grund welcher Untersuchungen und Tests Nähr- und Gesundheitswert beurteilt werden. Dann wird bei den obengenannten Nahrungsmittelgruppen besprochen werden, welche Maßnahmen mit einer Minderung, welche mit einer Erhöhung des Nährwertes einhergehen und wo eine Beurteilung des Einflusses auf den Nährwert noch nicht klar vorzunehmen ist, so daß weitere Forschung angezeigt erscheint.

I. UNTERSUCHUNGSVERFAHREN UND TESTS ZUR BEURTEILUNG VON NÄHR- UND GESUNDHEITSWERT

Vitamine

Tierexperimente bzw. Stoffwechselversuche am Menschen haben zweifellos den zuverlässigsten Aussagewert, denn sie sagen nicht nur etwas über den Gehalt an einzelnen Vitaminen, sondern auch über ihre Ausnutzbarkeit und Verwertung aus. In den meisten Fällen gibt aber auch die chemische Analyse eine genügend klare Auskunft über die Einwirkung von Be- und Verarbeitung auf den Nährwert, insbesondere dann, wenn man am gleichen Nahrungsmittel Analysen vor und nach der Verarbeitung ausführt. Wenn man den Gehalt an einem besonders empfindlichen Vitamin, etwa der Ascorbinsäure, verfolgt, ist dies zugleich ein guter Test für Erhaltung von Nährwert und Qualität im allgemeinen. Wenn Vitamin C nicht zerstört oder durch Auswaschen verloren gegangen ist, kann man annehmen, daß auch die Faktoren, die das Lebensmittel ansprechend machen, gut erhalten geblieben sind: Nämlich Aroma, Farbe, Duft und Form sowie Gehalt an allen hitzeempfindlichen Nährstoffen. Dieser Schluß beruht auf der Tatsache, daß Vitamin C nicht nur außerordentlich leicht durch Oxydation zerstört wird, sondern daß es unter allen Vitaminen und Mineralstoffen zu den am leichtesten löslichen Stoffen gehört.

Eiweiß und Aminosäuren

Eine Beurteilung des Nährwertes nur auf Grund der chemischen Analyse ist hier schwieriger als bei anderen Nährstoffen wie etwa Vitaminen und Mineralien. Die bei der Verarbeitung einwirkende Erhitzung führt bekanntlich häufig zu Veränderungen im Sinne der Maillard-Reaktion: Der nach Säurehydrolyse erfaßbare Aminosäuregehalt ist nicht verändert; trotzdem kann der Nährwert herabgesetzt sein, weil ein Teil der Aminosäuren nicht mehr der enzymatischen Hydrolyse zugänglich ist. Derartige Veränderungen sind dann erst im biologischen Versuch durch Bestimmung von Wachstum und Verdaulichkeit erfaßbar. Nicht immer muß dabei die Verdaulichkeit in vivo bestimmt werden, häufig geben auch –vor allem bei Vergleichsuntersuchungen—Verdaulichkeitsversuche in vitro einen guten Aufschluß über Verarbeitungseinflüsse.—Die Maillard-Reaktion führt aber auch zu neuen Produkten, die teils wichtig für Farbe und Aroma sind, teils unerwünscht sind.

Beeinflussung spezieller Funktionen

Sowohl bei den Vitaminen, vor allem aber bei den Aminosäuren können in manchen Fällen Einflüsse der Be- und Verarbeitung nur durch einen biologischen Test erfaßt werden. Es kann sich sogar die Bearbeitung eines Nahrungsmittels auf spezielle Funktionen auswirken, ohne daß man weiß, welche Bestandteile des betreffenden Nahrungsmittels dabei betroffen sind. Hierfür nur 2 Beispiele, auf die später noch ausführlich eingegangen wird:

Bei der weißen Ratte läßt sich mit verschiedenen industriell vorbehandelten Getreideprodukten eine stärkere Zahnkaries auslösen als mit unbehandeltem Getreide.

Das Wachstum von Mehlwurm-Larven (Tenebrio molitor) wird durch Mais und Maiskeime gehemmt. Beim Erhitzen wird der unbekannte Hemmstoff zerstört, die Larven wachsen dann genauso gut, wie sie auf Weizen und anderen Getreidearten außer Mais immer gedeihen.

In beiden Fällen ist bisher unbekannt, auf welche Inhaltsstoffe sich der Verarbeitungseinfluß auswirkt.

II. DIE EINZELNEN LEBENSMITTEL

A. Fleisch und Fleischprodukte

Nährwerterhaltung. A. ZUBEREITUNG. Bei schonender Verarbeitung oder Zubereitung ist eine Nährwertminderung unbedeutend. In Versuchen an Ratten konnte ein Unterschied im Wachstumswert zwischen frischem, gekochtem und gepökeltem Schweinefleisch nicht gefunden werden (103). In vitro-Versuche zeigten zwar eine Herabsetzung der Verdaulichkeit von gegrilltem bzw. geschmortem oder gebratenem Fleisch gegenüber rohem (45), insbesondere war bei unverminderter Löslichkeit der Abbau zu niedermolekularen, durch Trichloressigsäure nicht mehr fällbaren Peptiden (bzw. Aminosäuren) nach Kochen oder Braten verlangsamt (39), doch waren weder biologischer Wert noch Verdaulichkeit in vivo beeinträchtigt (67). Auch der Aminosäuregehalt war bei verarbeiteten Fleischprodukten (Würstchen, gepökelten Fleischwaren u.a.) nicht geringer als in frischem oder gekochtem Fleisch (89). So dürfte der Standpunkt berechtigt sein, daß man – jedenfalls auf Grund von Versuchen über Wachstum und N-Retention von Ratten—von einer Beeinträchtigung des Eiweißnährwertes bei der Zubereitung von Fleisch nicht reden kann (12).

Der Vitamingehalt von Fleisch zeigt dagegen eine deutliche Minderung schon bei der küchentechnischen Verarbeitung, wie Tabelle 1 zeigt.

In ähnlicher Größenordnung liegen auch die Werte anderer Autoren, die z.B. für Thiamin Kochverluste bis zu 43% (1) und für Pyridoxin zwischen 30 und 60% angeben (56). Selbstverständlich ist der Thiaminverlust abhängig von Dauer und Höhe des Erhitzens. Zahlenmäßige Belege hierüber ergeben sich aus Versuchen, in denen nach Erhitzen von Schweinefleisch in verschlossenen Gefäßen bei verschiedener Temperatur die Zeit bestimmt wurde, bis zu der 50% der im frischen Fleisch enthaltenen Thiaminmengen zerstört waren (79).

TABELLE 1. *Vitaminverluste von Fleisch bei Be- und Verarbeitung in % der im rohen Fleisch enthaltenen Vitamine*
(nach R. Hamm (38))

Fleisch	Vitamin B_1	Vitamin B_2	Nikotinsäureamid	Pantothensäure
Gut durchgebratenes Rindfleisch	28–30	13–27	9–35	14–31
Handelsübl. sterilisiertes Fleisch	30–40	0–10	0–10	20–30
Dehydriertes Fleisch	24–37	0	8	27–32
Gepökelter Schinken	10–20	0–3	0–5	
Frankfurter Würstchen	2–14	0	3–5	
Braunschweiger Wurst	19	5	11	
Salami	19	0	0	

Temperatur:	49°	54°	77°	82°	93°	99°	121°
50% Thiamin zerstört nach Stunden:	200	75	16	6,4	3,4	2,4	0,6

In Anbetracht solcher Ergebnisse sind kürzlich mitgeteilte Befunde über die Thiaminerhaltung in "ham loaf", einer Mischung aus geräuchertem Schinken, magerem Schweinefleisch, geriebenem Brot, Ei, Milch und Zwiebeln, erstaunlich (101). Hier liegen die Werte in dem zubereiteten Gericht, selbst nach 4 Monate langer Gefrierlagerung und anschließendem Kochen, nur um etwa 5% unter den Frischwerten. Ob die Erhaltung von Thiamin wirklich in Mischkonserven besser ist als in reinem Fleisch, und welche Stoffe für die Stabilisierung verantwortlich sind, ist bisher unbekannt. Dies zu ermitteln, wäre zweifellos eine interessante Forschungsaufgabe.

B. EINDOSEN-STERILISIEREN. Beim Sterilisieren kann man den gleichen Effekt der Abtötung von Keimen durch kurzes Erhitzen bei hoher oder durch längeres Erhitzen bei niedrigerer Temperatur erzielen. Da die Abtötungsquote von hitzteresistenten Bakterien bei einer Temperaturerhöhung um 10°C fast 10 mal größer ist, während die gleiche Temperaturerhöhung den Thiaminverlust nur um etwa 10% erhöht, ist also die Thiaminerhaltung besser, wenn man kurze Zeit hoch anstatt längere Zeit niedriger erhitzt (31).— Daß im Interesse einer besseren Vitaminerhaltung ein Sterilisieren in kleinen Dosen—infolge des Wärmegefälles nach der Dosenmitte zu!—günstiger ist als in größeren Dosen, zeigt folgendes Beispiel: Bei "pork-luncheon-meat" war der Thiaminverlust beim Sterilisieren in einer 340-g-Dose nur 25%, stieg jedoch auf 55% bei Verwendung einer 2,7-kg-Dose (31).

Beim Eindosen ist—im Gegensatz zu dem mehr schonenden küchenmäßigen Erhitzen! — auch der Eiweißnährwert von Fleisch gefährdet. Schon Beuk *et al.* (3) hatten den verminderten wachstumswert und

die herabgesetzte Verdaulichkeit von längere Zeit sterilisierten Fleischproben nachgewiesen. Folgende Tabelle 2 aus einer neueren Arbeit (102) bestätigt die damaligen Ergebnisse. Ratten erhielten entweder frisches Schweinefleisch oder Fleisch, das verschiedene Zeiten (15, 30, 60, 120, 195 oder 220 Minuten) auf 120° erhitzt war. Für Wachstum und Eiweißausnutzung ergaben sich die in Tabelle 2 niedergelegten Werte.

Daß die Verminderung des Eiweißnährwertes—wenigstens zum Teil!—auf eine Herabsetzung der enzymatischen Spaltbarkeit der Proteine bzw. auf verminderte Resorption der Aminosäuren zurückzuführen ist, ergibt sich aus Aminosäurebestimmungen im Blut der Vena portae: Bei jungen Ratten lagen eine halbe Stunde nach der Verfütterung von rohem Schweinefleisch die Aminosäurewerte um 100 bis 340% höher als die Ausgangswerte. Hatte man dagegen Fleisch verfüttert, das 4 Stunden im Autoklaven erhitzt war, blieb der Anstieg der Werte unter 100% oder wurde ganz vermißt (102).—Ähnlich wie Fleisch verhält sich auch Fisch (9).

C. BESTRAHLUNGS-STERILISIERUNG. Das vielversprechende neue Konservierungsverfahren, die Anwenddung ionisierender Strahlen, ist in weitem Ausmaß auch bei Fleisch erprobt worden. So liegen zahlreiche Literaturangaben über Stoffwechselversuche mit bestrahlungssterilisierten Lebensmitteln, insbesondere auch über Fleisch vor. In scheinbarer und wahrer Verdaulichkeit, in N-Bilanz und biologischer Wertigkeit konnten in Versuchen an Ratten Unterschiede zwischen rohem und bestrahltem Rindfleisch nicht gefunden werden (64). Viele Autoren sind auch der Ansicht, daß gesundheitliche Bedenken gegenüber dem neuen Verfahren nicht beständen. So konnte in Versuchen an 3 Generationen von Ratten, insgesamt fast 3.000 Tieren, deren Futter zu 60% der Kalorien aus bestrahltem Fleisch bestand, weder in der Nahrungsausnutzung, noch im Wachstum, in Größe, Lebensdauer, Fruchtbarkeit noch auch in der Zusammensetzung von Blut und Organen oder in dem Auftreten von Tumoren eine Abweichung gegenüber Kontrolltieren beobachtet werden (76).—Ein noch länger dauernder Versuch (4 Generationen von Ratten, Versuchsdauer 2 Jahre), in dem Wachstum, Lebensdauer und Fortpflanzungsfähigkeit verglichen wurden, hatte das gleiche Ergebnis (8).

Ein günstiger Einfluß scheint sich auf die Verdaulichkeit zu ergeben: Die enzymatische Hydrolysierbarkeit (pepsin digest residue) war bei verschiedenen Fleischarten nach Bestrahlungs-Sterilisierung kaum oder doch sehr viel weniger herabgesetzt als nach Hitzesterilisierung, wie Tabelle 3 zeigt.

Vielfach wurde jedoch eine Zerstörung einzelner Aminosäuren nachgewiesen, in Rindfleisch vor allem Glutaminsäure und Serin; in Leguminosen sind Lysin und Arginin betroffen, Glutaminsäure und Serin dagegen gar nicht (siehe Tabelle 4; 97. 98).

Von den Vitaminen ist Thiamin besonders bestrahlungs-empfindlich (86). Die Abhängigkeit der Zerstörung von der Bestrahlungsdosis zeigt Tabelle 5.

Andere B-Vitamine, z.B. Pyridoxin, sind hier sehr viel resistenter (54). Dagegen ist bei mit bestrahltem Fleisch gefütterten Ratten ein Vitamin-K-Mangel beobachtet (65), dessen Ursache noch genauer Klärung bedarf.

Im ganzen sind die wissenschaftlichen Vorarbeiten für die Bestrahlungssterilisierung noch keineswegs abgeschlossen. Weder sind die Ergebnisse organoleptischer Untersuchungen voll befriedigend, noch ist zu übersehen, ob das Verfahren auch gesundheitlich völlig unbedenklich ist. Immer wieder einmal werden bei Versuchen Ergebnisse beobachtet, die in irgend einer Richtung ungünstig sind, deren Ursache man sich aber nicht erklären kann. Weitere ernährungswissenschaftliche Grundlagenarbeit ist daher notwendig.

TABELLE 2. (102) *Minderung des Eiweissnährwertes Durch Eindosen*

Druckerhitzung Zeit in Min.	Gew.-Zunahme in 4 Wochen	g Gew.-Zunahme / g Eiweiß	% Verlust an Eiweißnährwert
0	41,7±1,6	2,08	
15	41,0±2,3	2,05	
30	36,7±1,8	1,83	12
60	37,1±1,6	1,85	12
120	34,1±1,6	1,70	18
195	32,6±1,8	1,63	22

TABELLE 3. (91) *Enzymatische Freisetzung von Aminosäuren aus bestrahltem Fleisch, Konzentration von Aminosäuren (mg/g Protein) in Pepsin-Hydrolysaten*

Aminosäure	Truthahn			Schweinefleisch			Rindfleisch		
	Kontr.	2 × 10^6 rep	Hitze	Kontr.	2 × 10^6 rep	Hitze	Kontr.	2 × 10^6 rep	Hitze
Methionin	6,9	6,8	4,8	10,0	10,0	6,6	10,0	9,3	7,0
Cystin	1,04	0,99	0,52	1,4	1,1	0,44	1,1	1,1	0,32
Lysin	2,8	2,6	2,1	3,2	2,4	1,8	3,4	3,1	1,9
Tryptophan	4,2	3,6	1,5	5,2	4,6	2,0	7,0	5,3	2,1
Valin	10,6	10,8	6,8	11,5	10,6	6,9	12,4	11,4	7,0

Hitzesterilisierung: Truthahn: 116°C, 114 min.
Schweinefleisch: / Rindfleisch: } 116°C, 161 min.

TABELLE 4. (97.98) *Ionisierende Bestrahlung und Aminosäuregehalt von Fleisch und Leguminosen (g/100 g Eiweiß, N x 6.25)*

Aminosäure	Erbsen		Lima-Bohnen		Rindfleisch	
	unbeh.	2, 8 Mill. rad	unbeh.	2, 8 Mill. rad	unbeh.	2, 8 Mill. rad
Serin	4.74±.07	4.58±.16	6.25±.12	6.22±.04	4.42±.07	2.75±.06
Glutaminsäure	13.10±.12	12.90±.13	12.88±.14	13.01±.13	15.07±.13	8.15±.12
Valin	4.82±.02	4.85±.04	5.31±.02	5.48±.04	4.51±.05	4.43±.06
Tryptophan	1.52±.02	1.54±.02	2.60±.03	2.70±.02	1.32±.01	1.29±.03
Lysin	8.60±.06	7.10±.10	10.44±.05	9.21±.09	8.73±.11	8.46±.08
Arginin	13.46±.16	11.86±.14	7.57±.10	6.05±.08	6.90±.09	5.59±.08

TABELLE 5. (33) *Thiamin-Retention in unter N_2 bestrahltem Fleisch (Thiamingehalt in % der Ausgangswerte)*

Dosis	Schaf	Kalb	Schinken
0.5×10^6 rep	58	56	60
1.4×10^6 rep	33	44	41
2.0×10^6 rep	25	22	35
4.0×10^6 rep	17	11	16

D. TROCKNUNG-GEFRIERTROCKNUNG-GEFRIERLAGERUNG. Trockenfleisch soll haltbar sein, insbesondere nicht ranzig werden und möglichst geringe Verluste erleiden an Quellbarkeit-Schmackhaftigkeit-Nährwert. Es ist schwierig, daß ein Produkt allen drei Anforderungen genügt. Ein in Deutschland als "Camping-Kost" empfohlenes sehr schmackhaftes Produkt zeigte in eigenen, bisher unveröffentlichten Versuchen an Ratten im Vergleich zu Frischfleisch einen Wertverlust (Gewichtszunahme pro g aufgenommenen Eiweißes) von 20 bis 40%. Über einen so hohen Verlust kann auch die gute praktische Verwendbarkeit nicht hinwegtrösten. Das ideale Verfahren der Fleischtrocknung sollte eigentlich die Gefriertrocknung sein. Doch auch hier kommt es bei zwar erhaltenem Eiweißwert leicht zu Veränderungen der Proteinstruktur, so daß bei verzögerter Rehydrierung ein holziger, trockener Geschmack resultieren kann (37).—Sehr gute Gefrier-Trockenprodukte haben Schäfer und Grüttner (34. 87) von Fisch herstellen können. Die biologische Wertigkeit entsprach der von Casein, die Verträglichkeit war selbst für frühgeborene Säuglinge gut.—Tiefgefrieren und Gefrierlagerung sind ideale Konservierungsverfahren, doch gehen auch sie, wenn auch erst innerhalb vieler Monate, allmählich mit Veränderungen von Aussehen, Geschmack und Verdaulichkeit einher (68).

E. EXTRAKTION MIT LÖSUNGSMITTELN. Zur Extraktion von Fett werden nicht nur Ölfrüchte, sondern wird bisweilen auch Fleisch—insbesondere als vorbereitende Maßnahme in der Herstellung von Trockenfleisch—mit verschiedenen Lösungsmitteln behandelt. Gleichzeitige Dehydrierung und Entfettung mit Äthylendichlorid oder Propylen führt zu einer signifikanten Verminderung der Ausnutzbarkeit von Methionin (Wachstumsversuche an Ratten). Dies wurde jedoch nur bei Rindfleisch beobachtet, während die gleiche Behandlung bei einigen anderen Fleischarten nicht nachteilig war. Es ist also notwendig, den Einfluß technologischer Vorgänge auf den Nährwert bei jedem einzelnen Nahrungsmittel zu prüfen, Analogieschlüsse sind nicht ohne weiteres möglich (14).

Zusätze. Zugabe von *Antibioticis* hat keinen spezifischen Einfluß auf den Nährwert von Fleisch (53). Wenn man aber Fleisch von Schweinen, die Zulagen von Aureomycin oder Terramycin erhielten, an Ratten verfüttert, haben diese Tiere eine im Vergleich zu Kontrolltieren etwas verbesserte N-Bilanz. Auf das Wachstum der Tiere wirkt sich dies allerdings nicht aus (13).

Durch *Polyphosphate* kann man Fleisch älterer Tiere so quellfähig machen, wie es das junger Tiere von sich aus ist. In einer zusammenfassenden Stellungnahme von RUF heißt es hierzu (85): "Phosphate können, richtig angewendet, ein gutes Produkt besser machen, aber sie können kein schlechtes Produkt gut machen."—Gesundheitliche Bedenken gegen ihre Anwendung bestehen nach Schreier (88) nicht, denn alle Polyphosphate, auch die zyklischen, werden erst nach ihrer Aufspaltung, d.h. in Form von Orthophosphat, resorbiert und als solche auch in den Harn ausgeschieden.—In der Zusammenfassung eines Symposiums über "Kondensierte Phosphate in Lebensmitteln" (51) heißt es, daß kondensierte Phosphate sicherlich nicht giftiger seien als Orthosphosphate. Ihre Wirkungen seien eigentlich Orthophosphatwirkungen. Befürchtungen, daß der Mineralhaushalt (Calcium, Kupfer, Eisen u.a.) gestört werden könne, träfen nicht zu. Selbstverständlich muß auch die Zufuhr kondensierter Phosphate so gehalten werden, daß die Gesamt-Phosphatzufuhr sich im Rahmen des als optimal bekannten Ca/P-Quotienten von 1:1 bis 1:2 hält.

B. Getreideprodukte (Cerealien)

Nährwerterhaltung und Nährwertminderung. A. MÜLLEREI (MAHLEN, SCHÄLEN U.A.). Die bekannte Tatsache, daß das Mahlen des Getreides mit einem bei den verschiedenen Vitaminen recht unterschiedlichen Verlust verbunden ist, macht Abbildung 1 deutlich. Erhöhung des Gehaltes an einem Vitamin zeigt sich jedoch bei der Bearbeitung von Hafer: Der

TABELLE 6. (72) *Verschiebung des Aminosäuregehaltes in Abhängigkeit von der Ausmahlung*

	Bedarf des Menschen Nach Rose (g/Tag)	Weizenvollkorn (287 g)		75% Ausgemahlenes Mehl (297 g)		65% Ausgemahlenes Mehl (304 g)	
		g Aminosäuren	in % des Bedarfs	g Aminosäuren	in % des Bedarfs	g Aminosäuren	in % des Bedarfs
Valin	1,6	1,64	+2	1,60	0	1,75	+9
Isoleucin	1,4	1,49	+6	1,60	+14	1,56	+11
Leucin	2,2	2,86	+30	3,0	+36	3,03	+38
Threonin	1,0	1,33	+33	1,27	+27	1,26	+26
Phenylalanin	2,2	2,10	−4	2,38	+8	2,23	+1
Tryptophan	0,5	0,48	−4	0,34	−32	0,34	−32
Lysin	1,6	1,13	−29	0,94	−41	0,86	−46
Methionin + Cystin	2,2	1,67	−24	1,56	−29	1,67	−24
Gesamt	12,7	12,7		12,7		12,7	

Gehalt an dem biologisch aktiven α-Tocopherol liegt in Haferflocken um 30 bis 40% höher als in Rohhafer (80).—Zu Verlusten führt die Ausmahlung auch bei Mineralstoffen und Spurenelementen. Doch erlaubt hier der Gehalt allein keine Aussage über den Nährwert: Vollkornmehl enthält zwar mehr Calcium und Eisen als feineres Mehl, doch kann durch das im Vollkorn enthaltene Phytin ein Teil seiner Mineralstoffe der Ausnutzung entzogen werden. Be- und Verarbeitung von Getreide wirken also in gewissem Sinne nährwertverbessernd.

Im Gegensatz zum Gehalt an Vitaminen und Mineralstoffen nimmt der an Eiweiß und Aminosäuren mit der Ausmahlung nur verhältnismäßig wenig ab (72). Zur Versorgung mit der gleichen Gesamtmenge an essentiellen Aminosäuren ist von Feinmehl nur wenig mehr notwendig als von Vollkornmehl, wobei sich die einzelnen Aminosäuren jedoch verschieden verhalten (siehe Tabelle 6) (72).

B. BLEICHUNG. Mehl wird gebleicht einerseits zur Verschönerung des Aussehens, andererseits zur Verbesserung der Backfähigkeit.

Die durch Oxydation wirkenden Bleichmittel können die Zerstörung von Nährstoffen oder die Bildung toxischer Produkte verursachen. Das sich unter NCl_3-Wirkung bildende Methioninsulfoximin führt bekanntlich zu dem als "Hundehysterie" beschriebenen Krankheitsbild. Wenn auch eine toxische Wirkung auf viele Tiere und auch auf den Menschen nicht vorzuliegen scheint, hat man dennoch aus berechtigter Vorsicht heraus in vielen Ländern die Verwendung von NCl_3 verboten.—ClO_2 scheint zur Bildung toxischer Produkte nicht zu führen, Aminosäuren werden bei üblicher Dosierung nicht zerstört (63), auch im Gehalt an essentiellen Fettsäuren zeigt sich keine Abnahme (29), doch kommt es zu einem fast gänzlichen Schwund von Vitamin E (69). Dies ist umso schwerer wiegend, weil bei der Ausmahlung—im Gegensatz zu den B-Vitaminen—der Vitamin-E-Gehalt auch in weißem Mehl verhältnismäßig hoch bleibt (vgl. Abbildung 1). Andere Bleichmittel, z.B. Kaliumbromat, greifen Vitamin E zwar nicht an, führen aber zu Verlusten an B-Vitaminen (81). Wenn auch die Verwendung von Bromat und Persulfat—vom Bäckerekzem abgesehen—gesundheitlich unbedenklich ist (52), ist die Mehlbleichung mit diesen Stoffen doch als eine den Nährwert gefährdende Maßnahme in vielen Ländern, z.B. in Deutschland, verboten.

Die erwünschte Verbesserung der Backfähigkeit von Mehlen kann auch durch Verwendung von Ascorbinsäure erreicht werden (52. 29. 61). Allerdings ist es nicht gerechtfertigt, hier noch auf eine Vitaminwirkung hinzuweisen, denn diese ist praktisch nicht mehr vorhanden. Selbst bei Anwendung der empfohlenen Höchstkonzentration von 25 mg/kg Mehl käme zur Herstellung von 250 bis 300 Gramm Brot nur etwa 5 mg, d.h. weniger als 10% des Tagesbedarfs in Frage. Diese Zahl gälte aber nur, wenn die Ascorbinsäure erhalten bliebe. In Wirklichkeit geht sie bei der von ihr erwarteten Oxydation störender Mehlbestandteile—z.B. Glutathion zum Disulfid!—und durch die Backhitze praktisch vollständig als Vitamin verloren.

C. BEHANDLUNG MIT IONISIERENDEN STRAHLEN. Bei Getreide wird eine γ-Bestrahlung nicht—wie etwa bei Fleisch—zur Erzielung der Sterilität, sondern zur Abtötung tierischer Schädlinge angewandt. Die Strahlendosis kann daher entsprechend tiefer liegen. Die bisher vorliegenden Ergebnisse sind recht widerspruchsvoll. Teils werden keine oder nur unbedeutende Veränderungen im Gehalt an Wasser, Maltose, Rohprotein und verschiedenen Vitaminengefunden (16), teils Zerstörungen der Vitamine B_1 und E (49) sowie von

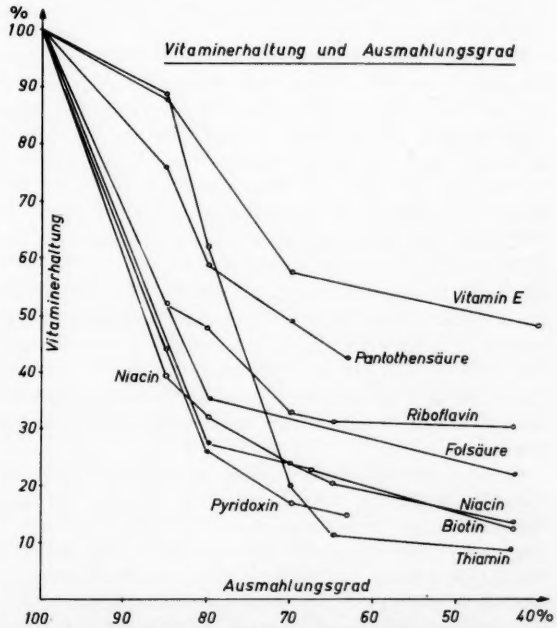

ABB. 1. Abnahme des Gehaltes an verschiedenen Vitaminen bei der Ausmahlung von Weizenmehl (nach Moran).

Vitamin A und Carotin (2). Einwirkungen auf die Stärke können Backfähigkeit und Qualität der Backwaren beeinträchtigen (50. 66). Andere Autoren weisen auf die—je nach Dosierung—auch mögliche Verbesserung der Backfähigkeit hin (49). Uneinheitlich ist auch die Stellungnahme zu der gesundheitlichen Unbedenklichkeit des Verfahrens. So finden Becker et al. (2) Unterschiede in der Fortpflanzungsfähigkeit bei Ratten, die ein zu 5/6 aus Weizen bestehendes bestrahltes Futter erhielten: Die weiblichen Tiere der Gruppen, die mit bestrahltem Futter gefüttert wurden, hatten in 3 Generationen weniger Würfe als Kontrolltiere. Luckey *et al.* (55) weisen hingegen darauf hin, daß sie in 3 Generationen von Mäusen keine Unterschiede beobachtet hatten, gleichgültig, ob die Kost mit Dampf, mit ionisierenden Strahlen oder gar nicht sterilisiert war. Die Kost war allerdings so zusammengestellt, daß eine genügend hohe Vitaminzufuhr gewährleistet war, selbst wenn durch die Sterilisation 98% der Vitamine zerstört worden wären. Auch die starke Wasserarmut des Futters mag für die günstigen Ergebnisse mit verantwortlich sein.

Für die Behandlung von Getreide mit ionisierenden Strahlen gilt hier also das gleiche, was schon im Kapitel "Fleisch" gesagt war: Vor der praktischen Anwendung des Verfahrens muß noch umfangreiche gründliche Forschungsarbeit geleistet werden.

D. BACKEN. Schon der erste Schritt des Backprozesses, die Teigführung, ist für die Vitaminerhaltung wichtig. Die B-Vitamine sind bei milder Gärung und einem Teig-pH von 5,4 bis 6,2 recht stabil. Bei höheren pH-Werten (ab 6,6) kommt es zu einer starken Zerstörung. Bei Zusatz von Na_2CO_3 und einem Teig-pH von über 6,6 kommt es zu einer vollkommenen Zerstörung von Vitamin B_1.

Die beim Backen einwirkende Hitze führt zu Zerstörungen von Vitaminen—vor allem B_1—und Aminosäuren—vor allem Lysin. Die Thiaminverluste liegen beim Backen von Brot bei 17 bis 23%, bei Kuchen—abhängig von der Verwendung von Triebmitteln—bei 23 bis 33% und bei Keks zwischen 26 und 30%. Rösten des Brotes führt zu weiteren B_1-Verlusten, so daß geröstetes Brot nur noch etwa die Hälfte des im Mehl vorhandenen Thiamins enthält (15). Für Lysin gilt ähnliches (5). Bei vitaminierten Backwaren werden die Vitaminverluste häufig *relativ* höher gefunden als bei nicht—vitaminierten (4), das zugesetzte reine Vitamin ist—vielleicht wegen des Fehlens von im natürlichen Vitaminträger vorliegenden Stabilisatoren—weniger resistent. Dieser Frage lohnt es sicherlich noch näher nachzugehen.

Gewisse Backprozesse können jedoch auch für die Vitaminversorgung vorteilhaft sein, wie das Beispiel des in Mexiko genossenen Gebäcks "Tortilla" zeigt. Hier ist es allerdings die dem Backen vorausgehende Alkalibehandlung von Mais, die zu einem Freiwerden von gebundenem Niacin führt. Beim Backen selbst büßen dann die Tortillas zwar noch etwa 30% ihres Niacingehaltes ein (6), doch ist der Genuß von Mais in dieser Form für die Niacinversorgung immer noch besser als der Genuß von Maisbrei ("Polenta").

E. SONSTIGE HITZEBEHANDLUNG. Getreidekeime werden zur Stabilisierung häufig einer trockenen oder feuchten Hitzebehandlung unterworfen. Hierbei kann es zur Zerstörung von Vitamin E und Carotinoiden bis zu 50% kommen (84).

Eine andere "Hitzeschädigung" bei Mais und bei Maiskeimen liegt darin, daß die nur in diesem Getreide vorkommenden, das Wachstum von Mehlwurm-Larven (Tenebrio molitor) hemmenden Stoffe durch Erhitzen zerstört werden. Durch Extraktion mit 0,25 nH_2SO_4 läßt sich ein nicht dialysables, hitzeempfindliches Konzentrat gewinnen, das bei einer Zulage von 1,6% zum Futter das Wachstum der Mehlwurm-Larven hemmt. Näheres über die Struktur dieser Stoffe ist noch nicht bekannt.

Eine sehr beliebte industrielle Behandlung ist bei verschiedenen Getreidearten, insbesondere bei Mais, die Herstellung von "flakes". Schon vor einem Jahrzehnt wies Mitchell (67) darauf hin, daß hierbei der biologische Wert des Mais-Eiweiß mäßig und seine Verdaulichkeit stark herabgesetzt ist. Die Beliebtheit der "cornflakes" hat durch diese Hinweise nicht gelitten. Da man Getreideflocken im allgemeinen mit Milch verzehrt, ist diese Nährwertminderung praktisch zu vernachlässigen.

F. INDUSTRIELLE VORBEHANDLUNG UND ZAHNKARIES. Das erhebliche Ansteigen der Karies in den letzten 100 Jahren und das vermehrte Auftreten dieser Krankheit bei Naturvölkern bei Übergang zu "Zivilisationskost"—z.B. Eskimos—gab Veranlassung zu der Vermutung, daß die Be- und Verarbeitung der Nahrungsmittel mit an der Karies schuld sei. Versuche von McClure et al. (57) mit verarbeiteten Getreideprodukten an der als Kariesmodell hervorragend geeigneten weißen Ratte stützten diese Vermutung. Die Möglichkeit, daß die teilweise Zerstörung von Lysin die Ursache sein könnte, wird dadurch gestützt, daß Zulage von 2% L-Lysin zu kariogenen Getreide-Futtermischungen das Ausmaß der Karies senkt. Daß andererseits Lysinmangel nicht grundsätzlich eine Kariesursache sein kann, wird durch eigene Versuche bewiesen: Verfütterung von verschieden lange erhitztem Milchpulver führt in allen Versuchsgruppen zu Karies gleichen Ausmaßes, obwohl die am längsten erhitzten Milchpulver eine Lysinzerstörung von 27% sowie Inaktivierung von weiteren 45% aufwiesen (Cremer *et al.* (22).

Nährwertverbesserung durch Be- und Verarbeitung. A. TEIGFÜHRUNG. Richtige Teigführung ist nicht nur wichtig zur Nährstofferhaltung, man kann durch sie auch eine Nährwertverbesserung erzielen. Die in vielen Getreidearten vorhandene Phytase—nur Hafer und Mais sind arm an diesem Ferment!—baut bei zweckmäßiger Teigführung einen wesentlichen Teil des Phytins ab, so daß es die Verwertung von Ca und Fe nicht mehr gefährden kann (13. 60).

B. BEHANDLUNG MIT WASSER. Bloßes Aufquellen von Gerstenschrot in Wasser und anschließendes Trocknen

kann zu deutlicher Nährwertverbesserung beitragen, wie in Versuchen an Hühnchen gezeigt wurde (30). Praktische Anwendung findet eine ähnliche Behandlung von Weizen und Reis in der menschlichen Ernährung: Die Herstellung von "parboiled wheat" oder "parboiled rice". Beim Quellen dringen wesentliche Anteile der wasserlöslichen Vitamine, besonders Thiamin, aus den äußeren Bezirken in das Innere des Korns, so daß die Vitaminverluste beim Schälen bzw. bei der Ausmahlung erheblich geringer und die Gefahr eines Vitaminmangels herabgesetzt ist.

C. BEHANDLUNG MIT ALKALI. Bei Mais führt selbst 40-stündiges Einweichen mit anschließend 1-stündigem Kochen nicht zu einem Freiwerden der gebundenen Nicotinsäure. Selbst nach 5-stündigem Kochen sind nur etwas mehr als 10% frei geworden. Bei Verwendung von 1% Kalkwasser (3 Teile zu einem Teil Mais) wird durch 50 Minuten langes Rühren bei 80°C jedoch die gesamte gebundene Nicotinsäure in die freie Form übergeführt (10). Diese ernährungsphysiologisch außerordentlich bedeutsamen Erkenntnisse—im Mais liegt fast die gesamte Nicotinsäure in gebundener Form vor und ist in dieser Form nicht ausnutzbar (48)—sind schon lange Erfahrungsgut der praktischen Ernährung in Mittelamerika und Mexiko: Obwohl hier Mais bis zu 80% der aufgenommenen Kalorien liefert, ist Pellagra hier nicht so häufig wie in anderen Maisgegenden. Bei der Bereitung des hier genossenen Maisgebäckes, der

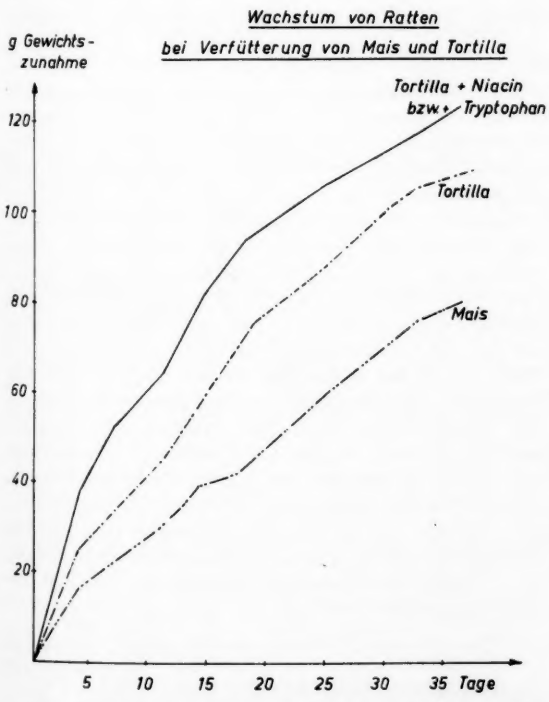

ABB. 2. Wachstum von Ratten, die Mais bzw. alkalibehandeltes Maisgebäck (Tortilla) als einzige Eiweißquelle erhielten (nach Carpenter et al.).

TABELLE 7. (92) *Anreicherung von Mehl. Für den Zusatz bzw. (bei USA) für den Gehalt—vorgeschriebene bzw. empfohlene Mengen*

Land	Zusatzstoffe pro kg Mehl:					
	Vitamin B_1 mg	Vitamin B_2 mg	Niacin mg	Vitamin D IE	Eisen mg	Calcium g
Chile	4,3	1,3	13,0	0	13–26	1,3
Dänemark	5,0	5,0	0	0	30	2,0
England	2,4	0	16,0	0	16,5	1,4
Schweden	4,0	1,25	40,0	0	30	0
U. S. A.	4,4–5,5	2,6–3,3	35–44	550–2200	29–36	1,1–1,4

"Tortillas", sorgt die Behandlung mit Kalkwasser für die Freisetzung des Niacins. Den Nährwert dieser Tortillas beweisen auch Rattenversuche, deren Ergebnis aus Abbildung 2 zu ersehen ist (10).

Anreicherung. A. ALLGEMEINES. Das Mißverhältnis zwischen der Bedeutung von Getreide als Grundnahrungsmittel und dem verhältnismäßig geringen Eiweißwert hat die Wissenschaft schon lange nach Wegen für eine Aufbesserung suchen lassen. Bei Mais schufen Pflanzenzüchter eine mit über 16% Proteingehalt ungewöhnlich eiweißreiche Art. Ihr wirklicher Vorzug wird allerdings dadurch geschmälert, daß die biologische Wertigkeit (Wachstumsversuche an Ratten) verringert ist. Wesentlichen Nutzen bringt sowohl bei dem proteinreichen wie bei dem proteinarmen Mais erst eine Anreicherung mit den Aminosäuren Tryptophan und Lysin (40) bzw. ganz allgemein die Anreicherung von Getreide mit Eiweiß oder Aminosäuren, Vitaminen und Mineralstoffen.

Die Geschichte der Anreicherung von Lebensmitteln mit reinen Substanzen ist mehr als 100 Jahre alt. Sie geht auf den französischen Chemiker M. Boussingault zurück, der 1833 vorschlug, jodhaltige Salze an die Bevölkerung von Kropfgegenden zu verabreichen bzw. dem Kochsalz Jodsalze beizumischen (36). Vor 50 Jahren wurde in Deutschland erstmalig eine Anreicherung des Brotes mit Calcium empfohlen (27). Umfangreiche Anwendung fand die Anreicherung erst in den letzten 15 Jahren. Aufklärende wissenschaftliche Veröffentlichungen, in denen die Notwendigkeit der Anreicherung durch Hinweise auf die Gefahr qualitativer Unterernährung dargelegt wurde, erschienen in den verschiedensten Ländern, so in Belgien (11), Chile (77), Dänemark (99), Westdeutschland (27. 100), Ostdeutschland (96), Schweiz (75), USA (104) mit einem besonderen Hinweis auf die Philippinen (90). Einen guten Überblick über Art, Umfang und Regelung der Anreicherungsmaßnahmen in verschiedenen Ländern gibt Tabelle 7.

Beachtenswert erscheint für die Anreicherung der Grundsatz des Council of Food and Nutrition der American Medical Association:
Die Anreicherung sollte beschränkt bleiben auf billige Grundnahrungsmittel, die einen wesentlichen Platz in der Kost einnehmen. Aber auch bei diesen sollten die Zusätze der Nährstoffe nicht höher sein, als diese Stoffe

normalerweise in einem derartigen Lebensmittel vorkommen (17).

Eine umfassende Übersicht über das Problem der Nährwertaufbesserung durch Anreicherung von Nahrungsmitteln unter Auswertung der bis 1957 zu dieser Frage erschienenen Publikationen geben Menden und Cremer (58, 59), so daß viele Einzelheiten hier nicht gebracht zu werden brauchen. Es seien deshalb hier nur einige wenige Arbeiten bzw. etwaige neue Gesichtspunkte erwähnt.

B. ANREICHERUNG VON GETREIDE, MEHL UND BROT. *Anreicherung mit Aminosäuren.* Auf die Bedeutung von Lysin und Tryptophan als die in erster Linie die biologische Wertigkeit von Getreide begrenzenden Aminosäuren weisen Culik (25) und Rosenberg (82) hin. Bei mit Lysin angereichertem Weißbrot würden 5 Scheiben dem Eiweißnährwert eines Hühnereies entsprechen, während man ohne Anreicherung 9 Scheiben rechnen müßte (58). Jahnke und Schuck (44) erwähnen die günstige Wirkung der Lysinzulage zur Vermeidung von Fettablagerung in der Leber, und Müller und Hötzel (71) weisen auf Grund von Versuchen an Ratten auf die günstige Aufbesserungsmöglichkeit der Gerstefütterung bei Schweinen durch Lysin hin.

Anreicherung mit reinen Vitaminen. Die Ergebnisse von Ernährungsversuchen mit jungen Ratten zeigen, daß mit B_1 und B_2 angereichertes Mehl sogar dem Vollweizen etwas überlegen ist (35).

Anreicherung mit Keimen. Da Getreidekeime die Hauptmenge der Vitamine und ein hochwertiges Protein (78) enthalten, besteht großes Interesse daran, sie zur Anreicherung zu verwenden. Der praktischen Anwendung der Keim-Beimischung zur Vitaminaufwertung heller Mehltypen sind jedoch gewisse Grenzen gesetzt: Aus praktischen Gründen kann kaum mehr als 1% Keimsubstanz zugesetzt werden. Hierdurch kann man einen Thiaminzuwachs von nicht mehr als 0,01 bis 0,02 mg/100 g Mehl erwarten, eine praktisch nicht ins Gewicht fallende Aufbesserung (62).

Anreicherung mit anderen Eiweiß- und Vitaminträgern. Trockenmilch, Trockenhefe, Sojamehl (21. 23), fettfreie Milch-Trockenrückstände, Lactalbumin (42), bestimmte Trockengemüse, die eine passende Aminosäuregarnitur aufweisen (93), sogar Reismehl, dessen Eiweiß im biologischen Wert höher liegt als der von Weizen und Maismehl (94) und schließlich Kleieextrakte (95) wurden auch in letzter Zeit wieder zur Anreicherung empfohlen.

Stoffwechselversuche am Menschen. Schon vor mehreren Jahren konnten Cremer *et al.* (24) in N-Bilanz-Versuchen am Menschen zeigen, daß die biologische Wertigkeit des Eiweißes von Weißbrot durch Einbacken von Lysin oder aber durch gleichzeitige Zugabe von Lysin in anderen Nahrungsmitteln erheblich verbessert und teilweise bis in die Größenordnung tierischer Proteine gebracht werden konnte. Kürzlich wurde von Bressani *et al.* (7) gezeigt, daß bei Wiederauffütterung eiweiß-verarmter Kinder mit einer Weizenprotein enthaltenden Kost eine ausgeglichene N-Bilanz und völliges Wohlbefinden erst erzielt werden konnte, wenn man Aminosäuren zulegte. Lysin erwies sich hierbei erwartungsgemäß als die die biologische Wertigkeit in erster Linie begrenzende Aminosäure.

C. ANREICHERUNG VON REIS. Daß Reiseiweiß einen etwas höheren biologischen Wert hat als das Eiweiß von Weizen und Mais, wurde schon erwähnt (94). Dennoch ist auch Reiseiweiß wie Getreideeiweiß allgemein deshalb nicht vollwertig, weil es nicht genügend Lysin enthält. Optimaler Wachstumswert bei Ratten kann erst durch Zulage von 0,34% Lysin (0,425% L-Lysin HCl) und 0,18% L-Threonin (0,36% DL-Threonin) erzielt werden (26. 83).—Die durch Lysin + Threonin erzielte Wachstumszunahme kann noch verbessert werden, wenn man zusätzlich Antibiotika, etwa Aureomycin oder Penicillin gibt (74).

Noch wichtiger als die Eiweißaufwertung ist bei Reis die Vitaminierung, insbesondere die Anreicherung mit Thiamin. Da, auch in primitiven Völkern, die Hausfrau nie darauf verzichten wird, den Reis zu waschen und weil beim Kochen von Reis ein Teil der wasserlöslichen Vitamine in das Kochwasser übergehen könnte, muß man die Vitaminierung in einer Form vornehmen, die dem Wasser Widerstand entgegensetzt (104). Der Erfolg der Verabreichung eines so behandelten Reises an die Bevölkerung der Philippinen ist aus folgenden Zahlen ersichtlich: Mortalität bei Beginn der Anreicherungs-Aktion 263/100.000. Innerhalb von 2 Jahren ist die Mortalität auf 28/100.000 gesunken, ein weiteres Jahr später waren überhaupt keine Todesfälle an Beriberi zu verzeichnen. Woods (105) weist darauf hin, wie dieses Experiment die Bedeutung einer bewußten Anwendung von Ernährungsprinzipien auf Gesundheitsfragen zeigt, er stellt den Versuch in seiner Bedeutung neben andere hygienische Maßnahmen wie Impfung, Insektenbekämpfung, Wasserreinigung und Milchpasteurisierung.

Kindernährmittel. Zur Sicherstellung einer ausreichenden Vitamin-D-Versorgung ist es vielfach üblich geworden, die—meist auf Getreidebasis hergestellten!—Kindernährmittel mit Vitamin D anzureichern. Es gibt Autoren, die der Ansicht sind, daß bei Kindernährmitteln die Überwachung der D-Vitaminierung einfacher sei als bei Milch. Sie meinen, daß bei üblicher Dosierung trotz gleichzeitiger dauernder Verwendung von vitaminierter Milch und vitaminierten Kindernährmitteln keine Gefahr der Überdosierung bestände (70). Viele Kinderärzte sehen aber doch die Ursache einer Reihe von Störungen –Lightwood-Syndrom!—in einer chronischen leichten Überdosierung von Vitamin D. Dieser Frage sollte man ernste Beachtung schenken, damit nicht therapeutische Maßnahmen in ihr Gegenteil umgekehrt werden.

C. Nährwertverbesserung durch Be- und Verarbeitung von Leguminosen (Bohnen und Erbsen)

Da sich an Hülsenfrüchten besonders gut zeigen läßt, wie sich Be- und Verarbeitung günstig auf den Nährwert

auswirken können, seien zum Abschluß dieses Referates hierfür einige ganz kurze Beispiele gebracht: In rohen Lima-Bohnen finden sich wachstumshemmende (47) und in rohen Gartenbohnen (kidney-beans; phaseolus vulgaris) toxische Stoffe (43), deren Zerstörung durch Hitzebehandlung Voraussetzung für die Genießbarkeit ist. Daß dies auch für den Menschen gilt, zeigten die zahlreichen Gesundheitsstörungen nach Genuß von Bohnenflocken—durch Walzen flach gequetschte Bohnen—, wenn sie kürzer als notwendig, nämlich nur 15 Minuten, gekocht waren (32). Verarbeitung ist hier also Voraussetzung für den Genuß!

Ein Beispiel für die günstige Wirkung industrieller Vorbehandlung auf den Nährwert liefern Versuche aus unserem Laboratorium (41): In Versuchen an Ratten (Wachstum und N-Ansatz) wurden verglichen:

Handelsübliche Erbsen (Kochzeit 2 Stunden)

Industriell unter Dampf vorbehandelte Erbsen (Kochzeit 10 Minuten)

Erbsmehl (Kochzeit 1 Minute).

Die biologischen Wertigkeiten dieser 3 Erbsenpräparate verhielten sich wie 100:114:150, also ein deutlich besseres Ergebnis bei den 2 industriell vorbehandelten Präparaten.

Zusammenfassung

Be- und Verarbeitung von Fleisch, Getreideprodukten und Hülsenfrüchten können zu Nährwertminderung führen. Es sind nach Möglichkeit schonende Verfahren anzuwenden. Be- und Verarbeitung können aber bei diesen Produkten, insbesondere bei Getreide und Hülsenfrüchten, auch durch Anreicherung mit fehlenden Nährstoffen bzw. durch besonders zweckmäßige Verfahren zu Nährwertaufbesserung führen. Weitere wissenschaftliche Forschungsarbeit erscheint im Interesse der Gesundheit und Leistungsfähigkeit von Mensch und Tier notwendig.

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Nutritional aspects of the use of spices and flavourings

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SATISFACTORY NUTRITION DEPENDS not only on the quality and quantity of foods consumed but also on their taste and flavour. The presentation of tasty foods in an attractive manner is a culinary art and is physiologically important because stimulation of the salivary and gastric secretion depends to a great extent on the appeasement of visual and olfactory senses.

The use of spices and natural flavouring agents were known to the ancients from time immemorial. That this practice is still current indicates its wide appeal and possibly its usefulness, though the exact scientific manner in which this is brought about is not easy to explain. Some evidence is available that the employment of spices and flavourings in generous amounts may bring about gastrointestinal discomfort and irritation, at least in groups of people not normally prone to these additives in their daily dietary practice. A certain amount of tolerance is evident in different areas where people use significant amounts of these additives without any disadvantage, as compared to other areas where undesirable manifestations of irritation appear commonly on short-term usages. The question, therefore, of giving a correct picture regarding the nutritional aspect of these additives vis-a-vis their disadvantages must remain open until further experimental and human pharmacological data under well-controlled conditions are made available.

SPICES

According to the U. S. Food and Drug Administration, spices are said to be "aromatic substances used for seasoning of food. They are true to name, and from them no portion of any volatile oil or other flavouring principle has been removed." Generally, spices owe their property to their volatile oil content although this is not true for all. In some cases a fixed oil is mainly responsible for the effect while in other cases the effect is due to a number of components, such as, alcohols, esters, phenols and their derivatives, terpenes, organic acid, sulphur-containing compounds, alkaloids, resins, etc., present in them.

Spices directly may be of very little nutritive value to man since they are taken along with foodstuff only in small quantities. However, they add delicious flavour to the food and it has been seen that a well-spiced food is more easily digested than an insipid one (44).

Very little evidence of the influence of spices on gastric secretion is available in the literature (9, 21, 25, 56, 16, 68) and that is not confirmatory. As testing of gastric secretory response to substances like spices is bound to meet with multiple difficulties, variation in the result is unavoidable (1, 57, 67) and reliable data on the subject are difficult to obtain. Palomera (50) has carried out extensive studies on the action of spices on the gastric acidity, on appetite and on gastric mucosa in animals and humans. His findings will be discussed later.

Harth (26) has reported that spices neither raised nor lowered the acidity of gastric secretion and has stated that spices can be safely used to give variety of flavour to the diets prescribed for treatment of stomach disorders.

It has also been observed that the administration of spices to drugs in conjunction with histamine-in-beeswax abetted the incidence of histamine-provoked ulcers in those animals. The irritative changes produced by spices on the gastric and duodenal mucosa very likely account for this abetting action. This finding suggests that spices should be withheld from the diet of individuals with peptic ulcer or ulcer diathesis (10). Heupke (27) studied the effect of spices upon gastric secretion and found that some spices had no more effect than slightly modifying the volume and acidity of the gastric juice.

Pal (51) reported that spices, though rich in calcium, phosphorous and nitrogen, when added in small quantities to Indian dishes increase the flavour but have no effect on nutrition and growth. However, calcium and phosphorous thus consumed can somewhat offset the ill effects of mineral deficiency by keeping the blood composition normal in respect to those 2 minerals. The normal structure of the parathyroid gland is also maintained.

According to Amelung (2) digestion of protein is retarded slightly by 0.1 % of various condiments and spices but the retardation is less than with 0.5 %–2 % sodium chloride which is a normal constituent of food.

Kokas and his co-workers (34), while studying the

effect of spices upon the movement of the intestinal villi and the absorption of glucose, reported that spices accelerated the movement of intestinal villi in concentrations attained during digestive processes, both in dogs and in pigeons. The absorption of glucose is also accelerated.

Let us now discuss the individual spices.

Almond. The almond is the fruit of *Prunus amygdalus* Batsch. It contains the glucoside, amygdaline which is hydrolysed with water to get the aromatic constituent, benzaldehyde. The almond contains 90% benzaldehyde, and albumin and 1 or 2 globulins. The isolated globulins do not support normal growth in mice when the diet contains 18% or less of protein. Almond oil contains very little vitamin A; even with 20% in the food mixture, rats suffer from vitamin-A deficiency, but mice do not suffer except that there is a considerably lowered fertility (45). Rose and co-workers (54) however, consider the almond to be a fairly rich source of vitamin A but, in their opinion, it contains some harmful substances which prevent young rats from growing when they are fed a diet of almond meal, salt mixture and a little starch when the almond meal constitutes 81% of the diet.

The digestibility of almond oil in comparison to a cream was studied by Hartwell (28) who reported that almond oil is digested slowly.

As almond oil contains a considerable amount of benzaldehyde and is considered a flavouring agent, it will be appropriate to discuss its toxicity and biological properties. Caujolle *et al.* (11) determined the LD_{50} of benzaldehyde in mice and found it to be 1.02 g/kg body weight.

Hosino (29) observed that fasting blood sugar was somewhat increased by benzaldehyde, and that 0.31 millimoles benzaldehyde in tyrode solution decreased the tone and stopped contraction of isolated rabbit uterus and intestine (3). Benzaldehyde more or less strongly inhibited the activity of carboxylase (35).

Anise. Anise is the dried, ripe fruit of *Pimpenella anisum* Linn. The essential oils in anise are anethole (90%), methyl chavicol and anise ketone.

Anise oil (10 drops) caused a decrease in bile secretion, with an increase in bilirubin and cholic acid with no change in cholesterol (52). It has been reported that the estrogenic activity of anise oil is due to anethole (71). Anise oil and anethole inhibit the deposition of glycogen in the liver of fasted adrenalectomized rats by adrenal cortical extracts. This effect is reversed by increased level of extracts. Anethole blocks the effect of adrenal cortical hormones in reducing insulin sensitivity of adrenalectomized rats (12). Gattefosse *et al.* (22) found that anethole, when ingested, is eliminated without trouble. Secretion of acid was favoured by anise (30).

Cajuput. Cajuput is the leaf of *Melaleuca leucadendron* Linn. It owes its value to its cineol content which is about 50%. In white mice the minimum toxic dose of cineol is 0.5 ml; the maximum nonirritating dose is 0.3 ml of 10% emulsion. Large doses, to animals, produce excitement, rapid pulse, nausea, vomiting, dilatation of the pupil and fatal collapse (56).

Capsicum; (red pepper). This is the dried ripe fruit of *Capsicum annuum* Linn or a closely related species. It contains an intense bitter principle, capsicin, and essential oils. Red pepper also contains ascorbic acid (1170–2900 U), vitamin B_1 (from 0.5 to 1.2 mg/kg), citric and malic acid (23). Rats fed on vitamin A-free ration, and showing distinct symptoms of avitaminosis, regained their growth and recovered xerophthalmia after the food was changed to 10% red pepper ration (69). The vitamin A content of red pepper is 33 U/g (4). Tocopherol content in red pepper is 2.4 mg/100 g (17).

In studying the influence of *Capsicum annuum* on serum protein, it has been found that the blood serum protein of rabbits decreases after feeding of *C. annuum* but rises to its former level within 4 days (41).

The action of capsicin on laboratory animals and on patients has been determined by a number of chronic and acute experiments. The general order of toxicity was low. One cubic centimeter of a 1% solution administered intravenously to a dog caused a sharp fall in blood pressure which was followed by prompt return to the normal. Other characteristics described were an irritant action on the skin and mucous membrane, an increase in salivary secretion, a relatively slight increase in gastric secretion, an increase in gastric motility, a slight increase in the activity of isolated rabbit intestine and guinea pig uteri, a slight effect on the isolated frog heart and a variable effect on respiration. Chronic experiments demonstrate kidney damage and indicate the presence of vitamins A, B and C (15).

Red pepper stimulates gastric secretion by mild irritation and by psychic effect (36). According to Palomera (50), red pepper (0.5–1 g) failed to stimulate the pouch secretion of dogs when introduced into the stomach. Pepper in 1-g doses was found to produce no apparent effect on the acidity of the gastric secretion. A 20-mg dose, when placed in the mouths of human individuals, did not show any detectable influence on the appetite or on the acidity of the gastric secretion. When added to the diet of rats in amounts equivalent to moderate human consumption red pepper failed to produce any detectable change in the caloric intake.

Caraway. This is the dried ripe fruit of *Carum carvi* Linn. It contains an essential oil of which the chief constituents are carvone and carvacrol. Other constituents are D-limonene, dehydrocarvone. Carvone content is between 50–65%.

The administration of carvone, which increases vitamin C excretion, protects rats against the hypoprothrombinemia to a degree approximately proportional to the excretion of vitamin C (6). Longnecker (42) earlier reported that the average excretion value, as high as 16.5 mg/day, and individual values, as high as 20 mg, followed the feeding of 100 mg of carvone daily. Carvone (10 drops) caused a slight increase in bile secretion, bilirubin and cholic acid with no change in cholesterol (52).

The caustic action of carvacrol is less than that of thymol and is easily absorbed cutaneously and eliminated through the lungs (22). After peroral intake, 25–95% is absorbed in 24 hours. After absorption they are excreted in the urine very quickly and only slightly in faeces. This can be demonstrated in the blood, lungs, kidneys and liver only for a few hours after ingestion. None is stored in the lungs and even with large doses only traces are excreted in the expired air. This causes water retention in the liver as judged by the decreased solid content (58). Carvacrol is readily absorbed from the gastrointestinal tract and produces vomiting (probably by gastric irritation), diarrhoea, loss in weight and paresis. The fatal dose for animals is 0.1–1 g/kg body weight (56); the toxic dose is 0.3 g/kg. When the quantity injected reached 0.1/kg carvacrol produced apnea for a few seconds after which breathing was almost normal until death. Blood pressure showed a gradual decrease (20).

Cinnamon. This is the bark of the following 2 species: *Cinnamonum zeylanicum* Nees and *C. cassia Blume.* The bark contains an essential oil, of which the chief constituent is cinnamic aldehyde.

Cinnamon oil increased the nitrogen excretion in male animals (7). When 5–30 cc of cinnamon water were introduced into the stomach through a permanent gastric fistula, the tone of the stomach was markedly decreased, but only temporarily (53). Cinnamon oil accelerates and strengthens the peristaltic movement of rabbit's small intestine (59).

Clove. This is the dried flower buds of *Eugenia caryophyllata* Thumb. The essential oil in clove contains 90% eugenol, other constituents being furfurol and caryophylline. Clove oil apparently contains considerable amounts of antirachitic substances (60). Clove in 1-g doses did not produce any effect on the acidity of the gastric secretion. Clove in doses of 20 mg and placed in the mouth of human individuals did not show any detectable influence on the appetite or on the acidity of the gastric secretion. When this was added to the diet of rats in amounts equivalent to moderate human consumption, it failed to produce any detectable change in the caloric intake of animals. Clove (1 gm) failed to stimulate the pouch secretion of dogs when introduced into the stomach (50). Clove accelerates and strengthens the peristaltic movements of rabbit's small intestine (59). Aqueous emulsion of clove oil (5%) and eugenol (0.5%) yielded secretion with a mean pH greater than 8.0 (31).

A dosage of about 0.2 g/kg appears to be safe for experiments in dogs requiring orogastric administration of eugenol emulsion (43). Sober and co-workers (61) determined the toxicity of eugenol. The LD_{50} for pure eugenol administered to rats by stomach tube and without subsequent respiration, was estimated to be 1.8 ml (1.93 g/kg). The net impression of the effect of eugenol from the microscopic evidence was essentially that of circulatory collapse with resultant congestion.

Sober and co-workers (62) studied the response of gastric mucous barrier in pouch dogs to repeated topical application of eugenol. The authors concluded that the mucus collected from the gastric (corpus) mucus of Heidenhain pouch dogs following a single application of 5% aqueous eugenol emulsion for 15 minutes, is opaque, jelly like and whitish and contains only slight amounts of fluid suggestive of an inflammatory reaction. They observed that the mucous-secreting function of the dog's pouch is seriously impaired by 3–4 15-minute treatments with eugenol and that no more than 5 or 6 such applications are required for its fatigue or complete exhaustion. Function is quickly restored for 36 hours after the end of such a prolonged experiment. The mucosa gives appreciable evidence of renewed mucous secreting activity.

Eugenol administered subcutaneously and intravenously in single doses or successive injections has no influence on erythrocyte number, leucocyte number and the amount of blood pigment in the normal rabbit. Eugenol was anti-anaemic (63); it impaired mast cell diapedesis and gastric leucopedesis in the albino rat, and depressed the bacterial flora of the gastric juice (13).

Direct contact of a eugenol emulsion with the gastric mucosa resulted in gastritis and a 13% decrease in total gastric ascorbic acid. Systemic absorption of eugenol after injection into the duodenum, to prevent direct gastric contact, did not induce these effects. A decrease in adrenal ascorbic acid after eugenol administration suggests that a systemic stress effect is produced by the eugenol, distinct from direct gastric effect (8). A 5% eugenol emulsion stimulates secretion of gastric mucous without increase of acids. Three or four applications of eugenol to the gastric mucosa exhausts the mucous response (56).

Coriander. Coriander is the ripe fruit of *Coriandrum sativum* Linn. It contains an essential oil the principle constituent of which is linalol. Other constituents are d-linalol and pinene.

Gattefosse (22), while studying the physiological properties of linalol, found it to be a powerful anaesthetic. It is diuretic and eliminated through the kidneys and lungs. The lethal dose is generally about 3–4 g/kg body weight, death usually being caused by dehydration of the lungs by excess of oil.

Celery. Celery consists of the dried ripe fruit of *Apium graveolens* Linn. It has an essential oil, of which d-limonene is the chief constituent. Celery contains 1.15. g/kg of vitamin C (5). Celery in 3-g doses is reported to produce no apparent effect on the acidity of the gastric secretion (50).

Fennel. Fennel consists of the dried ripe fruits of *Foeniculum vulgare* Mill. Secretion of acid was favoured by fennel (30); when ingested, it is easily eliminated (22) and has a definite estrogenic activity (71). The essential oil of fennel accelerates or strengthens the peristaltic movement of rabbit's small intestine (59).

Fenugreek. This is the dried seed of the plant *Trigonellum foenum Graecum* Linn. It is apparently physiologically inert. Fenugreek has been suggested for the treatment of various forms of malnutrition in humans (49). Pow-

dered fenugreek strongly stimulates general metabolism and aids assimilation. Its medicinal use is indicated in rickets, scrofula and anaemia (32). It is an aromatic and considered carminative, tonic and aphrodisiac. Several confections made with this are recommended for use in dyspepsia with loss of appetite, in the diarrhoea of puerperal women, and in rheumatism. An infusion of the seeds is used in veterinary practice. Fenugreek seeds are very rich in nitrogen and phosphorus. The total protein in fenugreek flour amounts to about 36%, most of which is present as albuminoids. It has been reported that phosphorus in fenugreek exists as phytin, nucleo-albumin and lecithins. The proportion of lecithin varies between 1.55–1.7%. Although the seed is highly nutritous, the commercial article has a bitter taste and an unpleasant odour which later is communicated to the perspiration of the user thus rendering the seed objectionable as a food. Patients fed on the flour of the seed, stabilised by treatment with vapours of boiling ethyl alcohol, showed a very marked and rapid increase in weight (70). Oil extracted from seeds of fenugreek was shown to possess a lactation promoting factor in rats, and was active in doses of 1 mg/kg body weight (18).

Parsley. It is the leaves and dried ripe fruit of *Petrosellinum sativum Hoffm*, and is very rich in vitamin C (24). Its essential oil contains a considerable amount of apiol. The minimal lethal dose of apiol for mice has been reported to be of the order of 0.1 g/100 g body weight. It has been shown that intravenous injection of apiol provoked hypotension in dogs, cats and rabbits and increased respiratory rate and depth. The drug exerted a marked depressive action on isolated intestines and direct stimulating effect on isolated uterus. Small doses of apiole given orally or subcutaneously increased diuresis in rats; large doses diminished diuresis (46).

Sage. Sage is the dried leaf of *Salvia officinalis* Linn. It has been shown that sage in 3-g doses was found to produce no apparent effect on the acidity of gastric secretion (50). The oil has been reported to have spasmolytic and bactericidal properties and to diminish salivation (19).

Sweet marjoram. Sweet marjoram is the leaf of *Origanum majorana* Linn containing 3% of essential oil. It has been reported that in dogs the intravenous injection of 1 cc/kg body weight of a saturated solution of the oil in 33% ethyl alcohol is regularly followed after 30–45 minutes by a marked increase in the peristaltic movements of the intestine, particularly of the ileum. Administration by stomach tube of 3–4 drops of the oil per kilogram body weight produced the same effect after about 1 hour.

FLAVOURING AGENTS

Flavouring agents considered here are those aromatic plants or parts of the plant which contain sapid or odorous principles and can be used in foodstuffs. The flavouring agents used in foodstuffs are generally one of the essential oils or one or more of organic compounds contained in such oils which give the characteristic flavour. The flavouring agents can be divided into 2 groups, the essential oils derived from natural sources and the pure organic compounds. These latter may be either synthetically prepared in the laboratory or isolated from existing natural sources.

The merits and demerits of some of these principles have already been considered under spices which constitute the main ingredients. Some of the remaining ones are discussed below. It may be incidently mentioned that the data on these are very scanty and whatever data are available in the literature have been taken out for consideration.

Citral. This can be obtained from the oil of lemon grass. It is also present in the oil of lemon peel. Citral at 13–115 mg daily dose produces fatty infiltration in rats, and causes local ulceration in guinea pigs. When injected subcutaneously, citral raises blood sugar in rabbit and leads to galactosuria after galactose feeding. The results, in the opinion of the author (64), are of importance in the use of food flavouring substances. Following the administration of citral at 0.029 g/kg the blood fructose content is increased and fructosuria follows (37).

Ethyl acetate. It has been reported (47) that for goldfish the lethal concentration in water is 1.1–1.2% for ethyl acetate. When injected subcutaneously in rats the lethal dose is 5 g/kg. Chronic poisoning by inhalation produces pulmonary oedema and cloudy swellings of the kidneys, liver and myocardium. It decreases the tone of isolated smooth muscle. When injected intravenously in rabbits the first effects were hypertension and increase in respiration. Rabello (55) reported that ethyl acetate exerted a depressing action on isolated guinea-pig intestine. Bijlisma (7) while studying the effect of ethyl acetate on nitrogen excretion, reported that dogs can be given 4 cc/kg ethyl acetate without injury. A 10-cc dose is lethal. Salivation, somnolence and deranged coordination are the symptoms. It has been reported that oral administration of ethyl acetate in a daily dose of 13–115 mg causes fatty liver infiltration in rats and local ulceration in guinea-pigs (64).

Geraniol. Geraniol is the chief constituent of the essential oil extracted from *Pelargonium odoratissimum* Ait. It has been reported that the phosphatase activity of dog liver extract was unaltered by treatment with dilute alcoholic solution of geraniol (33). It has been shown that geraniol is diuretic and is eliminated through the kidneys and lungs. The lethal dose is generally about 3–4 g/kg body weight, death is usually caused by the dehydration of the lungs by an excess of geraniol (22).

Menthol. Menthol is present to the extent of 56% in the essential oil of *Mentha piperata* Linn. It has been reported that an injection of menthol to an animal on a dog-chow diet showed high reducing capacities of the liver and gut (65). Kerr and Levy (38) have reported that after the intraperitoneal injection of menthol in vegetable oil into mice, glucoronidase increased in the liver but not in the kidney. Gross damage was observed in the liver but not in the kidney. Schmid (66), while studying the toxicity of menthol, reported that it is

quite toxic to frogs with depleted glycogen reserves. In well-fed frogs, menthol was detoxified by conjugation with glucoronic acid even after removal of the liver.

Thymol. This is present in Ajowan (*Trachyspermum ammi* Benth) oil to the extent of 40–50%. It is also present in *Moriarda didyma* Linn and *Thymus Zygis* Linn. Thymol in 1-g dose to man, caused no symptoms except a sensation of warmth in the stomach. Large doses produced dizziness, severe epigastric pain, excitement, soon followed by nausea, vomiting, marked weakness, drowsiness, quick soft pulse, tinnitus and deafness, salivation, sweating, then collapse with cyanosis, fainting and coma. Toxic dose of thymol has been reported to be of the order of 0.15 g/kg (20). Injection of thymol to animals on a dog-chow diet showed high reducing capacities of the liver and gut (65). It has been reported that thymol showed inhibitory action on the intestine, depressed the tone of both longitudinal and circular muscles (39, 40). After peroral intake of thymol 25–95% is absorbed. After absorption it is excreted in the urine very quickly and only slightly in faeces. It causes water retention in the liver as judged by the decreased solid content (58). Thymol (10 drops) causes no change in the bile secretion, bilirubin and cholesterol (52). Moller (48), while studying the effect of thymol on thyroid gland noticed that prolonged administration of thymol to guinea pigs produced signs of activation in thyroid gland without causing an increase in oxygen consumption.

Vanillin. Vanillin can be obtained from *vanilla planifolia* Andrews. In some species of vanilla it is present to the extent of 1.69 to 2.75%.

Caujolle (14), while studying the inhibiting action of serum cholinesterase by essential oil, found that when added in the proportion of 1:10,000 to horse serum containing acetylcholine, the following essential oils inhibited the action of the serum cholinesterase: peppermint oil—60%; lavender flower oil—58%; sage oil—37%; thyme oil—36%; marjoram oil—35%; lemon grass oil —34%; rosemary oil—13%. Menthol acted like peppermint oil. Thymol had a much stronger action than thyme oil.

CONCLUSION

An account has been given above of the nutritional, biochemical and toxicological aspects which might have an indirect bearing on nutrition of some commonly used spices and flavouring agents. Admittedly, the data available are not adequate to make a categorical statement regarding the nutritive value *per se* of any one of this type of food additives. However, their value in consumer appeal and in increasing palatability of foodstuffs with its associated stimulus on the gustatory and olfactory sense organs and possibly some degree of psychic and psychomotor stimulation cannot be ignored.

Herein may lie the real value of spices and flavourings in human and animal dietary, though this value may be of an indirect nature. The addition of a colouring agent may have only aesthetic value and consumer appeal but the spices and flavourings as additives stand on a different footing. Without being individually a nutrient, spices may help in preservation of foodstuffs and in its presentation to the consumer in a manner which appears to have a positive influence on appetite stimulation and gastrointestinal physiology. No experimental evidence is available indicative of their synergistic or additive effects in improving nutritive value of foodstuffs. Neither is there any evidence that the mere presence of spices may act as catalytic agents in the metabolism of any particular essential ingredient of food. Remotely, it may be postulated that in the fresh state (probably less in dried and stored forms), these may have some effect on mineral metabolism. Undoubtedly some enrichment of the food in an unexplainable way occurs. More exhaustive studies in animal and human pharmacology and nutrition are called for in this extremely interesting field of study.

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Nutritional aspects of the use of food colors

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THE COLORING OF NATURAL PRODUCTS is one of Nature's most magnificent achievements. It is therefore only logical that from earliest recorded history man has been attempting to duplicate or improve upon her efforts. This interest and concern regarding the appearance of all things about us follows naturally enough from the fact that 'sight' is our most important sense and has a greater effect on our reactions and behaviour than any of the other senses. However, man has not stopped at the attempt to improve the appearance of his surroundings but has also been striving to make his food more attractive.

We know for example that the ancient Egyptians of 3500 years ago enjoyed confections that were highly colored. A little later in history it is reported that the soldiers of Alexander the Great of Macedonia, on returning from a campaign in India, brought with them a sweet reed called 'khand.' It was mixed with spices and honey and was also highly colored. Our word candy is derived from this Hindu word 'khand,' although it has come to us by way of the French 'candi' meaning candied. Until the middle of the 19th Century, little progress was made in the coloring of foods owing to the fact that the only colors available were mineral pigments and colors extracted from animal and vegetable sources. However Sir William Perkin's synthesis of 'aniline purple' in 1856 opened up an entirely new field which, over the past century, has developed into a highly specialized industry with literally thousands of synthetic dyes at its disposal. Only a limited number of these are employed as food colors.

In order to determine the colors which were actually being used in foods, the World Health Organization in 1956 asked its members, numbering 85 at the time, for information regarding legislation on food colors currently in effect in their respective countries. A total of 64 replies were received. These indicated that 43 countries had permitted lists of food colors including 114 different synthetic compounds and approximately 50 natural colors. The remaining 21 countries either had a short prohibited list or no legislation. I have examined these lists to determine to what extent the colors used in the United States and Canada were employed in other areas. I found that Amaranth, FD&C Red No. 2 appeared on 34 of the 43 permitted lists; Erythrosine, FD&C Red No. 3 on 33, and Naphthol Yellow S, formerly FD&C Yellow No. 1, on 32. The numbers gradually dropped until Yellow AB and OB, formerly FD&C Yellows No. 3 and 4, respectively, were permitted in 14 countries and Oil Red XO, formerly FD&C Red No. 32, in 8. It is of interest to note that the last 3 colors which were obviously not in good standing in most countries, are no longer being certified for food use in either the United States or Canada. It does provide a ray of hope that eventually we may be able to achieve a uniform list of food colors throughout the world. I should hasten to add however that there is no immediate prospect for what would obviously be a very desirable state of affairs.

The volume of synthetic food colors employed in the United States amounts to approximately 1.6 million pounds per annum, while the corresponding figure for Canada is approximately 150,000 pounds. The amount of colors derived from natural sources is small compared with these values. I have been asked to discuss the nutritive value of these products. As you are well aware, the vast majority of these colors have no nutritive value whatever. However there is one notable exception and that is β-carotene. This compound is widely distributed in nature—the most important sources are carrots, palm oil, and alfalfa. However the successful synthesis of β-carotene was achieved in 1953 and since 1954 this compound has been in commercial production. In the United States, between 6000 and 7000 kg of both natural and synthetic β-carotene are used in foods per annum. Each gram of β-carotene has provitamin A activity of 1,666,700 IU. Since the recommended intake for the normal adult in the United States is 5000 IU/day, according to the Food and Nutrition Board of the National Research Council, the amount used in foods would satisfy the requirements for Vitamin A of approximately 6 million individuals. I think you will agree therefore, that the use of β-carotene to color foods does result in a significant contribution to human nutrition. This is on the assumption of course that there is no destruction of the β-carotene during the processing or storing of the food. In most products such losses are insignificant, although in baked goods the losses may range from 5 to 25%.

β-Carotene in its pure form consists of reddish-brown glistening crystals which are insoluble in water and only slightly soluble in alcohol and most organic solvents.

Therefore it has been necessary to develop forms which are suitable and convenient for incorporation into foods. The products recommended for use in the coloring of fat-base foods have been described by Bauernfeind *et al.* (1). The problem of limited solubility has been solved by a reduction of the crystal size and by the use of heat to prepare saturated oil solutions.

A product which is recommended for use in the coloring of margarine, shortening, lard, vegetable oils, process cheese, dried egg yolk and winter butter, prepared by the continuous process, consists of a liquid suspension of 30% micropulverized trans β-carotene in a vegetable oil. This product will pour at 70°–90°F but is semisolid at 40°–50°F. A further preparation for use in the same foods is a suspension in hydrogenated vegetable oil. It is semisolid to 90°F but will pour at 100°F or above.

There is also commercially available a β-carotene gel in vegetable oil which may contain from 1 to 10% of the coloring material and is recommended for use in the coloring of winter butter manufactured by the batch process and for the home coloring of margarine. When stirred or agitated, the gel structure is broken and the product pours readily. Riel and Johns (2) have reported favorably on the coloring of winter butter with β-carotene. They found that the vitamin A potency was increased, on the average, by 3800 IU/lb. It requires an amount of β-carotene equivalent to 5000 IU of vitamin A to provide a satisfactory color for margarine. The amount required for other foods varies with the intensity of the color desired.

Water-base foods require a further modification of the coloring agent (3). The problem of producing a dry form of β-carotene which was stable, water dispersible and of a practical potency was solved when it was discovered that a supersaturated solution of high concentration produced by dissolving β-carotene in hot vegetable oil would not crystallize from solution if it was rapidly emulsified before crystallization started. The resulting emulsion was then converted to dry beadlets. This product has been recommended for use in beverages, ice cream, yellow baked goods, confections, puddings, soup mixes, gelatin desserts, and similar products.

There is another aspect of the use of color in foods which I should like to discuss briefly, and that is the influence of color on our reaction to foods. Foster (4) has discussed a number of these factors in an excellent paper on the psychological effects of food color. He points out that psychology is concerned with such special provinces of behaviour as perception, motivation, emotion, learning and thinking. He expands on these various factors as related to our choice of foods as follows:

"1. Perception: Food selection or judgment of food quality would be extremely difficult if color discrimination was removed even though size, texture, shape and other cues were left intact.
2. Motivation: Food color and color of the environment in which the food is seen can significantly increase or decrease our desire or appetite for it.
3. Emotion: Liking or disliking a food is conditioned by its color; attractive foods are sought out as pleasure-giving, while unattractive foods are avoided as probably painful.
4. Learning. By the process of experience, we learn what colors to expect and consider "natural," and we predict rather precisely what properties a food or beverage will have from our memory of similar materials.
5. Thinking: Our reaction to unusual properties or to new foods can be changed if they are explained to us."

Foster (4) points out that there have been very few scientific investigations in this area. This opinion has been supported by Young (5) who states that as far as food preferences and reactions to food are concerned, white rats, racoons, pigs and chimpanzees have been exposed to scientific scrutiny about 10 times as frequently as man. However when man has been investigated, some rather amazing results have been obtained.

Bradley (6) has described an experiment in which 4 samples of sweet rolls were produced from the same formula containing a substantial quantity of frozen whole egg. The first sample contained no added coloring material but egg color was added to the other samples in increasing amounts. A taste panel showed a preference for the products containing the 2 highest levels of color, which was significant at the 0.1% level. In this case it was apparent that the color was the factor which increased the acceptability of these sweet goods. Foster (4) tells of an experiment in a large supermarket in which women were asked to wear colored goggles while making their selections. They were asked to complete their shopping and then to remove the goggles and examine all they had purchased. Every woman involved was surprised at some of her selections and many wished to return some of the goods. They experienced most difficulty with meats, cheeses, fruits and vegetables.

In another experiment (4) a number of foods, including meat, potatoes, peas, shrimp and mint jelly were served in pairs to a taste panel and the panel members were asked to indicate their preference. The foods were identical except that they had been colored with different amounts of vegetables dyes. One of the foods in the pair were colored to resemble the natural, normal or expected color of the food, while the other was an intensification of the normal hue. As might be expected the panel members preferred the food with the natural color but for the wrong reasons. Without exception they reported that the off-color foods had decided off-flavors.

I believe that these examples illustrate the point that in the use of color, the food manufacturer has a potent weapon which can strongly influence the choice of foods. It is at once apparent that the use of color could have either a good or bad influence on nutrition. If the consumer is attracted to a food because the color suggests the presence of a significant proportion of highly nutritious ingredients such as eggs or butter, when, in fact,

such ingredients are not present, the effect could be undesirable. On the other hand, if highly nutritious foods are made more attractive through the use of color then obviously the effect would be beneficial. Certainly the situation demands that processors use food colors with discretion.

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Panel Summary—Processing and Additives on Food

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WE CAN BEGIN OUR SUMMARY by referring to the questions that have been asked of Dr. Kon, who pointed out that the aura of sanctity that surrounds milk is probably not justified, and that milk suffers little, if any, from the kind of processing to which it is subjected, after being processed through the cow. His prophesy that in the future we will be consuming more processed milk in the form of dried milk rests, I think, on sound ground.

Dr. Kon was asked whether the position that milk occupies in the diet today, as a special food which must not be disturbed in any sense, is justified. His theory is that we must accept milk as it is furnished and reject that which can be avoided, but not otherwise to regard milk as warranting any special position in the diet. He pointed out that lysine availability is not disturbed in milk, there being no significant change in its biological value during processing, at least as far as pasteurization is concerned and probably very little in [further] drying. He was asked about dental caries and replied that any effect on dental caries from processing of milk is extremely remote. Dr. Kon cautioned against the indiscriminate fortification of milk with vitamins indicating that to the extent that indiscriminate fortification of foods generally is carried on, there are likely to be risks in excessive intake of certain nutrients, particularly vitamin D.

The question of sensitization as a result of penicillin residues in milk was raised. It seemed to be his opinion, that very little evidence exists to support this possibility, and, in any case, regulatory controls are being exercised. This applies also with respect to fallout residues.

As far as the effect of heat processing of milk on alleged growth hormone factors and enzymes in milk is concerned, Dr. Kon had no knowledge of what these growth hormone factors were, if any, and in the case of enzymes in milk, they probably serve very little purpose except possibly to the cheese maker.

Dr. Dols elaborated on the effect of processing on fats and oils, indicating that not only have tests been conducted in animals but long experience with refined, deodorized, and hydrogenated oils gives very little reason to believe that their nutritive value is significantly impaired. Such processed fats, he pointed out, are similar to normal fats in the diet. He suggested the possibility that hazards due to radiation fallout might be minimized by packing the milk and storing it long enough for the radioactivity to diminish to the point where it presents no hazards.

In response to the question regarding the use of antioxidants in the Netherlands, Dr. Dots said that three gallates have been permitted and BHA and BHT are now also permitted, as they are in this country. He reported the recently reported episode of the toxicity of a brand of margarine manufactured in the Netherlands, indicating the limitation with which he could discuss the matter now, being a member of an advisory staff to the Dutch Government. At any rate it seems clear that this was a new variety of a well-known brand of margarine, easily identifiable by its red wrapper, which was withdrawn promptly from the market when it was established that there was a causal relationship between a skin condition that developed in a large proportion of the population and the ingestion 10 days previously of this margarine.

Dr. Dormal stated that there seemed to be no evidence of injury to man from the proper use of pesticides, and pointed out the distinction between the toxic dose and the normal dose. She pointed out that there was no evidence of poisoning in man exposed to many times the levels of DDT that might occur as a residue from these pesticides on crops. She commented on the difference in the criteria for establishing safe levels of pesticides in this country and Canada on the one hand, and in Europe on the other. She concluded that this indicated a safer level of use in the European countries, to which I commented that I didn't think that differences of this magnitude had any real significance. The European tolerances cited are based on certain arbitrary assumptions as to the amount of vegetables and fruit that might be consumed. I indicated that tolerances in this country are based not merely on safe maximum levels but on the minimum levels necessary as a result of their use, if they are pesticides, or on the minimum levels necessary to produce the desired effects, if they are food additives.

Dr. Cremer reviewed the effect of various methods of processing on the nutritional value of foods. He said that the effect of heat treatment on the vitamin C content of food served also as an index of any changes in its flavor or other physical properties, including changes in certain other nutrients, vitamin C being the first to go; that the action of heat on the proteins in inducing the Maillard reaction might have some effect on nutritive value. So far as milk is concerned, there seems to be no indication of the loss of nutritive value in the processing, but the addition of lysine was reported to have reduced the incidence of caries in animals.

Dr. Mukerji reported on the apparent lack of effect of the high level of ingestion of the large number of spices and flavorings that are known to be consumed, particularly in India; however he indicated this is an area that needs more attention on the part of scientific investigators. There seems to be little reason, if any, to associate this high level of use with the incidence of either peptic ulcer or cancer in these areas. Chronic toxicity studies of the type that are currently required on food additives in this country have not been systematically conducted on spices. I expressed the view that this would be an important area for investigation, because these spices and natural oils, some of which are generally recognized as safe in this country, would present a formidable list of chemicals if we but knew the complete identity of their components. These are being revealed in increasing number today because of such techniques as gas chromatography.

Dr. Chapman reviewed the use of colors in various countries and suggested the advisability of uniformity in laws for purposes of international trade. He indicated the extent to which colors are used in the United States and in Canada and the importance of their use from the esthetic or psychological standpoint. There is little doubt that colors play an important nutritional role, perhaps not directly as nutrients, but certainly in making food more desirable and more acceptable.

I discussed some of the criteria for establishing safety and how our attitudes regarding food attitudes are based, in large measure, on psychological prejudices and semantic inhibitions that people have concerning chemically identified substances as distinguished from natural foods. Animal experiments have to be translated into human use conditions and the more or less arbitrary criteria that we use for that transition may be more exaggerated than are necessary from the standpoint of public health protection.

I pointed out that some of our concepts of tolerance levels, such as tolerance for additives at zero level are rather unrealistic from a chemical standpoint; that in any case these tolerance levels are based on arbitrarily acceptable sensitivity of chemical analytical methods.

Finally, I emphasized the importance of a sound scientific, rather than purely conjectural, basis for restriction against the use of chemicals, when they are alleged, without supporting evidence, to be potentially carcinogenic; that if we do not adopt a more realistic position, limit restrictions to either presumptively or definitely demonstrable evidence, we will exclude from our diets not only many common foods and food additives, but much of the variety and attractiveness of our present food supply, and we will obstruct the use of chemicals toward the production of a greater food supply for an increasing world population.

PANEL VI

Animal Nutrition and Food Production

Chairman: L. A. Maynard, USA
Moderator: D. P. Cuthbertson, Scotland

Problems of animal nutrition in underdeveloped areas

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IN THIS FIRST PAPER, the problem of animal nutrition and food production in underdeveloped areas will be dealt with in a general way. It consists in aiming at an adequately increased availability of animal protein foodstuffs among the populations throughout the world, with particular emphasis on those which are in the greatest need of it. The problem will be considered therefore from the angle of human nutrition.

We shall examine first what may be the closest possible appraisal of the supplies actually available, and to what extent these are short of requirements. The condition is liable to serious deterioration in the future if effective measures are not enforced, because of the influence of demographic pressure which is being felt throughout all parts of the world by a rapidly expanding population, particularly in the underprivileged areas.

Two major factors should therefore come under consideration: *a*) malnutrition, due to protein deficiency (chiefly animal protein shortage), and *b*) growing requirements, because of population expansion as well as increased life expectancy, an important additional factor in the highly developed countries.

Adequate measures to solve this problem may be divided into 2 categories: *a*) short-term programs of assistance not involving any fundamental change in the existing social setup of the underdeveloped areas; small increases in available animal protein products can do much to improve substantially the health conditions, where malnutrition is acute. Although limited in scope, but difficult however to achieve, such measures are not to be underestimated in importance; but because of the demographic pressure so strongly felt in these underdeveloped areas, proper planning cannot be limited only to such short-term programs. By the expression 'short term' we refer to immediately applicable methods of assistance. *b*) The need for long-term policies of sound agricultural planning is becoming urgently felt, in order to meet adequately larger scale requirements. There can be but little hope for achievement along these lines unless fundamental changes can be made to come about in the sociological setup of the underprivileged countries. Moreover large-scale international planning in food production and distribution must be foreseen between the 'under' and the 'highly' developed areas of the world.

PRESENT OVER-ALL SITUATION

FAO's investigations in the past (22) have indicated with sufficient accuracy that the population has about doubled in size during the 2 centuries between 1650 and 1850. It has doubled once again, but in half that period of time, namely in 1 century, between 1850 and 1950. It reached a figure of about 2500 million in 1956. It will probably rise above 3000 millions in 1975, and may reach 6000 million by about the end of our present century if nothing basically disturbing happens within the next 40 years to alter the slope of this curve. Only a small proportion, hardly one fifth of the world's entire population, is getting its proper share of food supplies (providing the people with the possibility of living at an optimal level of energy expenditure, namely some 2500 to 3000 calories per person and per day). The other 80% live at a much lower level, which may be reduced to about one half the one mentioned above, as is the case in India, and perhaps even less than that elsewhere (16–23).

Another factor is the extraordinary uneven distribution, in space, of human population in the various parts of the world. Whereas very densely populated areas are known to create problems of food distribution, extreme diversity is the characteristic feature elsewhere, namely in very large and underdeveloped regions, such as the African Continent.

There is a very striking map of Africa upon which maps of the U. S. A., China, India and Pakistan have been juxtaposed, having in between them African uncovered areas which, when blended together, correspond to surfaces that could easily include those of Europe (1). Africa, south of the Sahara, comprises 17.7% of the total land surface of the earth; it contained only 5% of the world population in 1954 and, at that same time, the African continent was estimated to include 44% of the world's potential power. Those are

the economic and geographical features which are characteristic of the situation in that important part of our planet and there are also climatic ones to be taken into consideration (1). Referring once again to the demographic pressure involved, let us also recall the fact that by the end of the present century, hence in 40 years' time, it has been anticipated that about 70% of the world population will be afroasiatic. For these various reasons I shall choose central Africa as a typical and outstanding example of an underdeveloped area of particular interest. It must not be forgotten, however, that the problems may not always be the same in all underpriviledged parts of the world.

Now, let us see how the situation presents itself with regard to the more specific question we are concerned with in this panel discussion, namely, animal protein food supplies. In the countries comprising the Western civilised world, people live on an average daily diet of some 100 g of total protein, 30 to 40% of which are from animal sources, hence an average consumption of at least 30 g of animal protein. This consumption level ranges somewhere between 15 and 30 g in many parts of the Mediterranean basin and also of the Latin American continent, whereas it drops below the 15 g level in Central Africa and in most parts of Asia (16–23). In the Congo basin and surrounding areas where some 12 million people are very widely scattered over extremely vast areas, mostly nonagricultural in the strict sense of the word, the 15 g level mentioned above, expressed in terms of meat and fish, would require a yearly supply of some 300,000 tons. In 1949, it was estimated that the actual available supply did not probably exceed some 20,000 tons (see table 2 for a more recent date). These few examples give an idea of the scope of the food problems confronting the world today.

FACTORS INVOLVED IN SOLUTION OF THE PROBLEM

It is customary to distinguish between *a*) economic and financial, *b*) agricultural and *c*) food technological angles of approach to the solution of the problem.

It should be stressed, however, that the sociological setup of the populations concerned is to be considered as one of the most essential factors of primary importance, although it has been usually insufficiently stressed (1, 4). This is what leads us primarily to distinguish between short-term and long-term programs of assistance. Moreover, it is also necessary to distinguish between 2 other aspects of the problem: *a*) Increased local production within the underdeveloped areas, and *b*) world-wide planning possibilities of food distribution between surplus areas of production and underpriviledged areas where requirements are more difficult to meet. It will be important also to weigh these 2 potential sources of agricultural supplies and to include in category *b*) a third possibility, that of finding other food sources in the future (non agricultural in the usual sense of the word), which could be anticipated owing to new technological developments hitherto unforeseen (4).

AGRICULTURAL PROBLEMS IN UNDERDEVELOPED AREAS

Short Term Programs of Immediate Assistance

Prospects for improvement are bound to remain within rather narrow limits. In such areas as Central Africa, food production takes place at the family unit level; the women are in charge of it. Farming is not a profession. The peasant is non existent. It would be a mistake however to underestimate what can be done under such conditions to improve the situation, particularly when food shortages are pronounced and malnutrition distressing. Health measures are of primary importance. The state of nutrition is closely related to infectious diseases and intestinal infestations, and both factors interact both ways. Education is obviously as important a factor as the economic level and purchasing power of the population.

Doctor Oomen's observations in New Guinea may be taken as a typical example of the effective measures that, in terms of nutrients, may seem to fall short of optimal requirements, but which have shown however striking results among groups of population whose health conditions had fallen close to the limit compatible with life, indicative of how much human nature can adjust in its struggle for survival (3). In his study of various groups of population he stressed the following facts. Some of these groups live on an average supply of some 1500 calories and 35 g of protein, part of which is derived from fish; their state of health is comparatively satisfactory in comparison to those living on an average diet of 1400 calories and 20 to 25 g of protein, in which case their condition was distinctly poorer than that of the former group. Kwashiorkor was however strikingly prevalent among other groups in which the protein intake was as low as 10 to 15 g with a higher calorie intake (1900 calories) because of a greater ration of carbohydrates. By slightly adjusting this balance in the diet and adding an additional 200 calories, including an extra 10 g ration of protein, strikingly good results were registered in due time.

Let us turn now to the over-all situation as it presents itself from continent to continent. Interesting data have been collected by N. Wright (65) regarding the ecology of domestic animals. In table 1 we have converted his data in order to stress the influence of 2 important factors: *a*) the space factor, showing how widely different the actual distribution of livestock presents itself in the various continents; and *b*) the relative importance of livestock in comparison to human population distribution within those continents (excluding Australia and New Zealand since these are, in fact, part of the Western civilised world).

Commenting upon this kind of information we shall see that its interpretation may be partly misleading, since the data for such large areas of the world, considering the very pronounced heterogeneity of their various areas, are expressed in average figures. Yet, we may point out certain characteristic features arising from

TABLE 1. *Human and Livestock Populations, 1935–39**

	Europe		North and Central America		South America		Asia		Africa	
	A	B	A	B	A	B	A	B	A	B
Human	75		7·4		5·1		42		5·6	
Cattle	21	28	4·2	56	5·9	118	8·5	20	2·5	45
Pigs	16	21	26	35	1·7	33	3·1	7·5	0·1	1·8
Goats	5·1	6.7	0·5	6.7	0·8	17	4·6	11	2·1	38
Sheep	24·3	32	2·5	35	5·4	11	4·7	11	3·5	63
Poultry	159	211	23·5	317	8·4	167	24	57	3·9	71
Equines†	5·1	6.7	1·0	14	1·3	27	1·2	2·9	0·4	7·6

A—Per 100 hectares (247 acres). B—Per 100 human population. * USSR not included in this table. † Includes horses, mules and asses (in Africa horses are only 25% of the total).

such general information. Let us emphasize first the well-known fact that to improve the proper balance of the over-all diet of a population by covering a larger proportion of its calorie requirements with food of animal origin is an expensive proposition both physiologically and economically. This is due to the fact that the yield, in terms of calories, produced by domestic animals as food converters is but 10 to 20% of the calories of the vegetable foodstuffs those animals consume. It is therefore much more economical to cover human requirements with staple foods of vegetable origin alone. This, however, cannot be done without prejudice to the proper balance in the diet of what has often been called 'protective nutrients.' Indeed, this proper balance can eventually be maintained with adequate vegetarian diets in which fresh vegetables and fruits can be supplied in sufficient quantity and variety, in which case, then, the economic aspect of the problem is no longer characterised by an inexpensive supply of the amount of calories required. An adequately balanced diet in all nutrients is more easily obtained from mixed diets, composed of foods from both vegetable and animal origin, than it is from purely vegetarian diets alone. Economically speaking, the same is also true. The problem in underdeveloped countries is therefore not only to improve the amount of calories provided in the food but also the balance of the diet by increasing the amount of available perishable foods of animal sources.

Cattle. Europe is very definitely the most densely populated part of the world, in terms of cattle. Africa is at the other extreme in this respect. On the other hand, in comparison to a given size of human population, the 2 American continents show the highest figures for cattle population. In Africa, the ratio of cattle versus human populations is, all in all, slightly higher than in Europe, but the general geographical dispersion of that livestock as well as the extreme heterogeneity of the different parts of that very large continent, are the main cause of the difficulties. As a matter of fact, 45% of cattle per 100 people may seem fairly high, but it must be remembered that livestock is most unevenly distributed throughout the various parts of Africa. Whereas a large proportion of livestock is found in South Africa (24, 39, 41, 56, 64), only a small amount is found in other areas, such as the desert regions of North Africa. If we compare, for instance, the situation in the Belgian Congo (B.C.) to that of the Ruanda-Urundi (R.U.), 2 contiguous areas in Central Africa, differing very widely however in size and in climatic and sociological conditions, the above mentioned ratios are very far apart, as indicated in table 2. R.U. covers a much smaller area than does the B.C., but it is much more densely populated, both in terms of people and cattle. The amount of cattle is about the same in both countries, but per 100 people, it is about 3 times greater, on an average, in the R.U. than in the Congo. In regard to the yield in meat production, during the same period 1956–57–58 it amounted to some 19,000 tons in the Belgian Congo and only to 16,000 tons in the Ruanda Urundi. It should be mentioned, however, that cattle breeding in the Congo was, until recently, mostly in the hands of a few European farmers.

Pigs. Pig breeding in relation to human population is an important factor in Europe and in the 2 American continents (North and South). In Asia, and more particularly still in Africa, it is less important. This is to be considered in relation to the fact that pigs, of all domestic animals, are the greatest contenders of man with regard to the agricultural products they consume. In certain areas, it may perhaps be possible, at the family unit level, to encourage limited breeding of pigs, but to do so with profitable yields requires highly developed and very efficient farming (see below).

Goats. Africa is the continent where the average population of this kind of livestock is the highest in relation to human population. This however is only an apparent advantage as a source of food, because goats among many African native populations are only bred as a sign of wealth or of social standing. Problems of sociological adjustments as well as educational campaigns are therefore involved, in the endeavor to encourage goat breeding for food purposes (both meat and milk).

Sheep. Europe, North and Central America, and

TABLE 2. *Information Drawn from Official Reports of the Belgian Administration, Ministry of the Congo 1958 and 1959*

	Belgian Congo			Ruanda-Urundi		
In millions	Human	Cattle*	Cattle/ 100 humans	Human	Cattle*	Cattle/ 100 humans
End of 1956	12.8	0.94†	7·3	4.48	0.93‡	20.7
1957	13.2	0.97	7·4	4.56	1.00	21.9
1958	13.5	1.01	7·5	4.69	1.01	21.6

* European and native farming. † That amount of cattle produced 19,000 Ton of meat in one year. ‡ That amount of cattle produced 16,000 Ton of meat in one year.

Africa are the main producers of wool and meat in terms of reference to the same size in human populations. But here again a single average figure for such a heterogenous and wide area, as the entire African continent, may easily be misinterpreted. In Central Africa, sheep are not to be considered as an important source of food.

Poultry. Europe and the 2 American continents in contrast with Africa, are the main parts of the world where poultry is an important source of food. In areas such as the Congo, the principles involved have been studied (10). Both with regard to meat and eggs, poultry can hardly be considered to play an important part in food supplies unless it is organised on a well-planned farming basis in areas where the very limited purchasing power of the population can be raised at the same time. The same is true also for animal protein foods in general.

We may conclude that some beneficial results can be obtained to improve food conditions with regard to meat production and distribution in underdeveloped countries at the family unit level of action. The perishability of meat adds to the difficulties of the problem, because it is chiefly consumed in the form of dried meat prepared in some sort of primitive way (sun dried). The same limitations hold true for eggs and poultry. As regard to milk, the possibilities are extremely limited. Even if production were possible, the problems of preservation and suitable distribution of such perishable products as animal protein foods would be the main limiting factors. In other words, the low purchasing power of the consumer is the chief obstacle. Imported dry skimmed milk distributed to well controlled health centers for consumption as liquid milk has done much to contend with malnutrition (relief schemes such as those of UNICEF for example). As a regular item on the food market however, rather than as a sort of medicine administered under medical and social welfare control because of lack of available drinking water, dry milk can hardly be expected to become a common article of diet, unless social, economic and public health conditions can be first very substantially improved. So far, it has only been in very rare and favourable circumstances that milk has been incorporated in staple food preparations such as cassava meal porridge, for instance, in certain industrial labor communities. But this has only been achieved in a very limited way.

Fish. Along the coast, in certain rivers and in lake districts fish is an important source of animal protein, most frequently distributed in the form of dried fish. Much has been done to improve fisheries, wherever possible in underdeveloped areas, even on an industrial basis in certain favorable instances. In more remote inland regions, and at the family unit level of production, a lot has also been done to develop soft water fish-pond projects. Great efforts were made in Central Africa along those lines, and indeed with encouraging results in various instances even when there were reasons for some doubt in regard to the possible success of such projects (12, 13, 32–34, 59–61).

Long Term Programs of Assistance Through More Ambitious Projects of Increased Food Production and Distribution of Animal Foodstuffs

Such programs become essential if one has to meet the consequences of increasing demographic pressure and not just fight malnutrition where it occurs most alarmingly. This problem is one of a much wider scope. The possibilities depend primarily upon the success in overcoming social obstacles. These are conditioned by an induced consciousness—if this can be brought about—among the populations concerned, of a requirement for a gradual change in the existing sociological setup, based on the concept of a need for a collective organisation of planned food production on a much wider scale than the existing one at the family level; a trend to be brought about by the coming into being of farming as a profession. Encouraging efforts have already been made to this effect in certain areas of Central Africa.

Such a fundamental sociological evolution could take place only if the populations concerned can be made to become conscious of such a requirement. In the future, the necessity of a change in the existing conditions must originate within these populations themselves. Indeed, it could not take place without outside influence and assistance, provided however the latter is requested from within the underdeveloped country itself. If one realises that that means the coming into being of a regular social revolution, one will realise how difficult and time-consuming it is going to be to get such projects started. In the past, when colonisation was the method used, the coloniser took the decisions, formulated plans which he imposed upon the colonised countries. Nowadays such methods have ceased to be an actuality and the world is still going to be confronted with a much more difficult problem to solve. Henceforth, the request for help and assistance will have to come from within the underdeveloped areas. It might possibly find a starting point in the form of pilot plan projects financed from without, in areas where potential financial resources, exclusively agricultural are limited; or partly at least from within, if local industrial potentialities exist and have already been exploited in the areas concerned. In both cases, however, the problem is primarily one of rendering the populations conscious of the necessity of such a trend for the sake of improving their own welfare.

Short of proper planning of projects, both in research and in production schemes, there is obviously but very little hope for substantial progress. Countries having 1 to 2.5 acres of cultivated land per capita of human population can produce all or nearly all of the various foods they need, but the quality of their diet, in particular with regard to meat and fat, depends on the efficiency of their agriculture and farming. Among the underdeveloped parts of the world, cultivated land per head of population is much smaller; in 1953 it was 0.8 acre in India, 0.5 in China and less than that in Japan, in which case the diet is vegetarian and inadequate. Two

thirds of the world population grow their own food and 80% of these live in vast rural areas where food is produced locally (56). Practically all over the world, agriculture operates below maximum efficiency. In 1950, there was less than 10% of the earth's land surface that was cultivated, and in the U. S. A., for instance, irrigation was only used from 35 to 55% of efficiency (7). It was estimated at that time that, if present knowledge was fully exploited, a world population of 6000 million people could probably be supported (7). Yet, important improvements have been registered in many parts of the underdeveloped areas of the world (23). In those parts, such as South Africa (24) and even in Central Africa, where difficulties are known to be great, one has succeeded in many ways: irrigation of grassland in areas of severe and constant drought, coupled with health measures necessarily linked with irrigation projects in order to prevent malaria from spreading where it failed to do so in the past because of lack of water in the areas concerned; intensive studies for improving or preserving soil fertility; rational farming; possibilities of raising food and feed crops (14); increased yield of meat and milk production (11, 24); improved grazing (49, 57); possibilities of yielding food and feed out of cash crops such as those producing protein as well as cotton concentrates, as an example; adequate livestock selection and breeding (26–31, 38, 40, 41, 46), including artificial insemination (52); and also livestock rational feeding (23, 37, 42–44, 47, 15, 52); livestock diseases in regard to infectious diseases, intestinal infestations, deficiency diseases, trace elements and toxic food constituents (6, 8, 9, 36, 47, 52, 63); staple food economics (39).

But most of the time such actions were primarily in the hands of the white man under the regime of colonisation, and adequate research and experimental stations were created by him for that purpose (example: IRSAC, Institut pour la Recherche Scientifique en Afrique Centrale; and INEAC, Institut National pour les Etudes Agricoles au Congo Belge, as well as similar institutions elsewhere, in Gambia for instance (Med. Research Council U. K.).

OTHER PROBLEMS

Nutritional Problems

These concern food problems of adjustments of inadequate diets, causes of malnutrition rather than undernutrition. They are linked with medical, social and economic problems. Vitamin or mineral deficiencies are included in this group. A voluminous bibliography exists on these problems which cannot be covered in this report. One single actual example will be mentioned simply for illustration (55). Nitrogen and sulphur deficiency due to improper balance between protein and carbohydrate supplies in the diet and also to defective quality of the protein consumed (lack of essential aminoacids) are other actual aspects among nutritional problems (kwashiorkor being a typical example (2)). Conditioned deficiencies form another classical group of nutritional problems stressing the close relation between nutritional and medical aspects. Increased availability of animal protein foods is the key to many of these nutritional problems.

Technological Problems

Improvement in high quality protein production is closely linked with various technological problems. We shall merely enumerate some of the most outstanding typical ones of present concern, since most of them are only indirectly concerned with animal protein production: *a*) Influence of livestock feeding on animal food composition, namely milk (45, 15, 48, 5). *b*) Increased production of high quality protein of vegetable origin, both as food and as feed. *c*) Food preservation and conservation. Reduction of food waste which is estimated to amount in bulk to some 20% of total world production (although it is much greater than that in many underdeveloped areas). *d*) Industrial production of dry animal protein foods, fish meal. The use of dry skimmed milk in farming. *e*) Justification for maintaining cassava cultivation notwithstanding its very poor nutritional quality. Economic aspect of the question. *f*) Search for new sources of high quality protein; microbiological synthesis; submarine resources; defreezing artic areas with a view to extend the world's agricultural domain (4).

Economic Factors

An important aspect of the problem of improving animal nutrition and animal food production in underdeveloped areas is the time factor. Considering the difficulties due to unfavorable conditions which prevail in those areas, and the increasing demographic pressure, one has to take into consideration what may reasonably be expected to correspond to realistic expectations. With this in view, one may examine what length of time has actually been required in those areas where the white man's 'know how' and the favourable physical conditions have both contributed to reach a state of high agricultural productivity. Several interesting statements are to be found in the literature in this respect. It has been anticipated that in 1980 the world's increase in population will need to increase the staple food production of today by at least 43%, and of the protective foods by an 88% (53). Attempts to evaluate the possibilities of increasing the supply of food and agricultural products by exploitation of new areas and increased yields have been made (7, 51, 56). In certain countries, namely in Europe, a maximum use and development of land has been achieved, although they are not completely self-supporting (35). But if we turn to areas where surplus food production potentialities exist, we have the case of Canada where it has been estimated that agricultural production could be increased by 50% and even more than that (54). There is the case of the U. S. A. where agricultural surplus production possibilities exist

in conditions of great flexibility (56). Moreover, certain parts of Latin American should also be mentioned. In New Zealand the high development actually reached in agricultural production required one century of intensive effort; farming covers 67% of the land; and it is anticipated that in 15 years' time the population will probably increase from 2 to 3 million people (+50%) and that an increase of 60% in livestock during that time is considered as a possibility (58). In Australia, where the climatic conditions and the soil are poor, and where water resources are limited, the present agricultural status was reached in about one century's time, and food is one of the country's export items (62).

The Union of South Africa is another example where agriculture is in the hands of the white man. Its agricultural domain covers 90% of the country, but there is vast room there for improvement in total production; consumption is increasing faster than production and, therefore consumption is at a low level, and animal protein food supplies are deficient (64); the soil Conservation Act has done much in 15 years' time to counteract misuse of the soil; the country is better adapted to pastoral rather than arable farming; there is a considerable lack of moisture. The country remains 40% short of the supplies it needs for human consumption (24, 25, 50).

CONCLUSION

It seems doubtful whether local production of food in adequate quantity and quality can be increased throughout all the underdeveloped areas of the world at a speed sufficient to meet, in due time, the requirements of the human populations of those areas, in view of the demographic pressure actually prevailing there. The potentialities are great, but the difficulties are at least as great. If there is hope for success in solving such important a problem, it should at least be considered as an extremely urgent one. In order to get anywhere near the goal, it seems indispensable to consider it also on a world-wide basis of adequate international, economic and technical planning. Surplus production, wherever possible, will have to be accelerated at a maximum possible speed, for purposes of distribution to the underprivileged areas.

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*This list is by no means meant to be a complete one, but merely a selection of books on the general subject or articles indicated as examples of the various points raised in this report.

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Efficiency of feed conversion by different classes of livestock in relation to food production

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THE EFFICIENCY of feed conversion or utilization is the ratio of output of animal product to the input of feed. In recent years this ratio or its inverse, feed required per unit product output, has been widely used as a convenient single figure whereby rations may be compared one with another, the value of feed additives and supplements measured and the merit of individual animals appraised as a first step in attempts to breed better livestock. The ratio has an additional appeal since the cost of feed is invariably the major cost in animal production, and feed efficiency calculations provide a simple means of assessing profitability.

The purpose of this paper is to compare different classes of livestock as convertors of their feed to human food. Many attempts have been made to do this, notable contributions having been made by Brody (19) and by Armsby and Moulton (2) in America and by Leitch and Godden (42) in Scotland. These and more recent estimates such as those of Mighell and Scoville (51) show considerable discrepancies when they are compared, in that some authors' conclusions appear at first appraisal to be quite contrary to those of others. For example, if the efficiency of the dairy cow is placed at 100, estimates of the efficiency of the pig vary from 94 to 182. For this reason no new calculations have been made here to add to what might appear confusion. Instead, an attempt has been made to account for these discrepancies, to indicate the determinants of efficiency, and to consider their application to the problems of meeting human needs for food.

Before dealing with the efficiency of different species as convertors of feeds to human food, some general aspects of the efficiency of food utilization deserve mention, and as illustration of the complexity of the problem, meat and milk production have been chosen.

MEAT PRODUCTION

The amount of human food in the carcass of a meat-producing animal can be expressed either as protein edible by man or as the calorific value of all the edible material including fat. Both amounts vary considerably with the age of the animal and with the amount of food which it has been given until it is slaughtered. The proportion of edible protein in beef animals, varying in weight from 120 to 1500 lb. and growing at very widely different rates, does not seem to vary much from a mean value of 8.5% of the live weight (61), though it declines to about 6% in aged animals which are fattened.

Calculations from dissection and analytical data show that a yield of 8–10% of edible protein is also true o sheep, poultry and pigs. Thus the live weight of a meat animal at slaughter is a fair approximation to the yield of protein edible by man. With fat, however, the proportion present in the live animal varies considerably, from 5% in young animals reared very slowly to over 40% of the live weight in old animals given considerable feed. It follows that the calorific value of all edible material in a meat animal follows a very different course with age to that of weight or protein. For a particular feeding regimen the curve relating calorie yield to age reaches a maximal rate later than does that relating weight or protein yield to age. It also approaches a limiting value much later in life than does protein. Furthermore, for different feeding regimens, curves relating calorie yield to age show much greater dispersion than do corresponding curves relating protein yield to age.

Part of the food an animal consumes is employed solely to maintain it, and it is only feed in excess of this essential need which is used to promote growth and fattening. Furthermore, the animal at birth has already incurred a considerable feed debt since its dam has been maintained and fed during her pregnancy.

It follows from the above considerations that for any particular feeding regimen there is an optimal age at which an animal should be slaughtered if the yield of human food divided by the total feed consumed, including that needed to produce the animal, is to be maximal. This can be referred to as the optimal age at slaughter, and it varies according to which criterion of human food output is adopted. If meat is regarded solely as a source of protein in the human diet, then since protein growth reaches its maximal rate early in life the optimal age at slaughter is considerably earlier than if calorie yield is taken as a criterion of worth.

During the last 100 years the age at slaughter of most of our meat animals has been markedly reduced and this reflects a reduction in the dependence of human populations on meat as a concentrated source of energy and an appreciation of its importance as a protein source. Even so, in industrial areas fat carcasses are demanded. The age at slaughter in practice takes into account consumer preference and may not be dictated by considerations of maximal efficiency of conversion of either protein or energy. This is seen in a more marked way where animals are slaughtered for processing. With bacon pigs and with broilers, factory processes are geared to carcasses of particular sizes. The age or weight at slaughter is then dictated by the need to provide a standard product for the consumer.

A second factor which determines the optimal age at slaughter in terms of yield of human food is the rate of growth of the animal, or alternatively its level of nutrition, for this is the main determinant of growth. The lower the feeding level and the smaller the growth made, the older should the animal be at slaughter to give maximal life time efficiency of feed conversion. The efficiency of conversion will, of course, be very much lower in a poorly fed animal than it is in a comparable animal grown quickly and slaughtered at either the same age or the same weight. Table 1 by way of illustration shows the effect of level of feeding on optimal age and weight at slaughter for a beef animal.

MILK PRODUCTION

To evaluate milk as human food is difficult for it supplies so many dietary essentials: a convenient measure is its calorific value. The amount of milk produced by cows expressed in terms of calories is dependent in part on the amount of feed they are given, and the relationship between annual feed intake and annual yield is one of diminishing returns (37). It follows that since there is a considerable feed cost in rearing cows to milking age, there is a level of feed intake during lactation at which life time efficiency of feed conversion is maximal. Most studies suggest that this optimal feed intake is slightly above current feeding standards particularly in the instance of those cows which are intrinsically high-yielding animals (9, 10). The higher yielding animal is naturally the more efficient at converting feed to human food and the range of efficiency of feed conversion in milking animals is particularly wide. Despite these wide ranges in individual efficiency and those consequent upon differences in the feeding levels adopted and length of productive life of cows, the economic profitability of milk production on the farm does not vary to a like extent because high-producing cows are more expensive to purchase than are low producers, and the cost of feed per unit weight increases with the level of feeding adopted.

It is possible that some of the discrepancies in the estimates of the relative efficiency of milk-producing animals and meat-producing animals stem from the fact that so many types of animal production are economically feasible, that the efficiency of these vary considerably and that economic factors and consumer preferences involve departures from optimal methods of converting resources to human food.

The above results imply that between-species comparisons based on the measurement of feed intake and product output of animals in a particular area may well reflect economic conditions rather than physiological factors. To compare the efficiency of human food production by different classes of stock an alternative approach is to consider the ways in which animals make use of feeds.

SPECIES DIFFERENCES IN ABILITY TO DIGEST FEED

A first stage in the above regard is to consider how well different species and classes of stock digest the same feed. The results of thousands of determinations of the digestibility of feeds have been analysed statistically, notably by Jarl (36), Axellson (6), Mitchell (52), Nordfeldt (56), Crampton and Rutherford (20) and by Glover and Duthie (30). In each species the apparent digestibility of the organic matter falls with increasing fibre content, and the major differences between species arise with respect to the magnitude of the fall per unit change in fibre content. Table 2 summarises a number of such statistical analyses and shows that feeds low in fibre are digested less well by ruminants than by pigs, but that as fibre content increases this

TABLE 1. *Weights and Ages at Which Cattle Should be Slaughtered to Maximize Efficiency of Feed Conversion*

Level of Feeding	Approximate Rate of Gain, lb/day	Criterion Edible Protein Yield		Criterion Energy Value of Edible Material	
		Live wt., lb.	Age, months	Live wt., lb	Age, months
High	2	840	13	1740	30
Low-normal	1	640	18	1100	36
Very low	0.6	450	24	800	48

Based on the data of Moulton, Trowbridge and Haigh (53, 54).

TABLE 2. *Apparent Digestibility of Organic Matter of Foods by Different Species in Relation to Fibre Content of Food*

Species	Percentage of Fibre			Depression of Digestibility/Percentage Unit Increase in Fibre
	0	15	30	
Ox	86	75	63	0.74
Sheep	89	76	63	0.86
Horse	88	69	51	1.25
Rabbit	97	74	51	1.55
Pig	94	70	46	1.60
Hen	86	57	27	1.96

Computed from equations of Mitchell (52), Axellson (6) and Nordfeldt (56).

situation is reversed. Then the most efficient are the ruminants, followed by the nonruminant herbivores, followed by pigs and finally, least efficient are poultry. There are no real differences between sheep and cattle and Piatkowski's (57) results show that the goat does not differ from other ruminants. These results indicate that fibre presents a barrier to digestion which ruminants, and to a lesser extent nonruminant herbivores, surmount but which pigs and poultry cannot. In this regard, however, Nordfeldt (56) has shown that the pig can digest the fibre of its diet, and that this ability increases with age. The increase in ability is associated with a simultaneous increase in the capacity of the large intestine relative to that of the rest of the gut, and the fibre is certainly digested by bacterial attack in the caecum and colon. Highly purified cellulose, free of encrusting polyuronides, is digested well by adult pigs, but its digestion is associated with considerable gas formation in the colon and caecum (63). Removal of the caecum reduces the ability to digest cellulose but it does not abolish it (45). With poultry, Bolton (14) has shown a similar change in the ability to digest cell wall constituents with increasing age. This was limited, however, to the pentosan fraction.

Fibre includes a large number of structural carbohydrates (see 34), and species differences in ability to digest the different carbohydrates from natural food sources is given in table 3. There is little doubt of the inability of poultry to digest cellulose and of their limited ability to utilize pentosan compared with the pig, and both these species are markedly inferior to the ruminants.

The same generalizations made with respect to species differences in the digestion of carbohydrate apply to protein. Calculations made from the results of Glover and Duthie (30) and Glover, Duthie and French (31) show that, given their preferred diets, simple stomached species are superior to herbivora and within herbivora the nonruminants excell. The depression of the digestibility of protein by fibre content is, however, considerably less in ruminants than it is in non-herbivorous stock.

TABLE 3. *Ability of Ruminants, Pigs and Poultry to Digest Carbohydrates of Natural Feeds*

Species	Type of Food	Digestibility of Carbohydrates, %				
		Starch or fructosan	Sugars	Cellulose	Pento-sans	Lignin
Cow	Hay good	96	96	82	79	Nil
	Hay medium	96	94	69	63	Nil
Sheep	Grass	100	100	82	78	Nil
Pig	Cereals	100	100	60–80		Nil
Hen	Cereals (mean values)	100	100	3	33	Nil

Compiled or computed from data of Bolton (13), Ely *et al.* (23), Blaxter and Graham (11) and Teague and Hanson (59).

FERMENTATION LOSSES

The ability of animals to digest cellulose and polysaccharides other than starch and simple hexose and pentose polymers resides in the activity of the bacteria inhabiting their gut. The end products of the fermentation are steam volatile fatty acids from formic to valeric, and on occasions lactic acid. Methane is also produced, together with heat. Apparent digestibility, determined as 100 × (feed-faeces)/feed, is thus not an approximation to the nutrients absorbed in those species where fermentation occurs. Table 4 summarises the approximate amounts of methane produced by herbivora and omnivora together with the concentration of steam volatile fatty acids in the contents of their guts.

The data for the CH_4 production of ruminants show variation from feed to feed, but even greater variation is found in pigs, indeed some workers have detected no methane formation in this species (28). The amounts produced by ruminants and herbivora are such that estimates of energy absorbed, based on the apparent digestibility of food, are at least 10% too high. Furthermore, the heat produced by the microflora is about 80% of the calorific value of the methane formed (48) so that the over-estimation in terms of energy is nearer 20% than 10%.

UTILIZATION OF ABSORBED NUTRIENTS BY DIFFERENT SPECIES

It has been one of the great unifying concepts in biochemistry that the final metabolic pathways of nutrient dissimilation and the methods whereby different species obtain free energy for work and synthesis of body constituents varies little from species to species (see 41). It might be expected that the ability of different species of animal to obtain free energy for the synthesis of body fat from a molecule of glucose actually absorbed as such is invariant. This appears to be true. Thus, table 5 summarises experiments in which glucose was given to ruminants and nonruminants and its ability to meet the free-energy demands of maintenance

TABLE 4. *Loss of Energy as Methane Gas by Different Adult Species and Concentration of Volatile Acids in Their Gut Contents*

Species	CH_4 Production kcal CH_4/100 g CHO Digested	g Steam Volatile Fatty Acids in Gut Contents/kg Body Wt.
Ox	60	1.50
Sheep	40	0.76
Horse	70	0.58
Pig	15	0.40
Hen	Nil	

Data computed from data of Axellson (7), Bratzler and Forbes (17), Swift, Bratzler, James, Tillman and Meek (58), Von der Heide and Klein (60), Breirem (18), Lehmann and Zuntz (43) and Elsden, Hitchcock, Marshall and Phillipson (22).

TABLE 5. *Utilization of Glucose as an Energy Source by Different Species*

Species	Efficiency of Utilization in Replacing Depot Fat as a Source of Energy for Maintaining the Animal	kcal Fat Synthesised per 100 kcal Glucose Absorbed
Sheep, abomasal infusion	95–100	71·5
Dog	94	68·9
Pig		71·5
Man	94	

From results of Armstrong, Blaxter and Graham (5), Lusk (47), Fingerling, Kohler and Reinhardt (28), and Fingerling and Schoenemann (27).

and to promote the storage of fat in the body measured. The results show that sugar was used by all species with comparable efficiency, whether as a source of energy to maintain the animals or for the synthesis of fat.

There is, however, little doubt that the steam volatile fatty acids, the end products of microbial digestion of feed, are utilized less efficiently than is glucose in the cycles of intermediary metabolism (3, 4). Hence the efficiency with which the energy of the absorbed nutrients is used varies from species to species according to the proportion of steam volatile acids in the end products of digestion.

In other words, ruminant animals and nonruminant herbivora, while having the ability to digest feed constituents which are unavailable to animals with simple digestive tracts, necessarily utilize the energy they obtain from feed with a lower efficiency.

NUTRITIVE VALUE OF THE SAME FEED IN DIFFERENT SPECIES

Table 6 summarises scattered experimental data which enable comparisons to be made between species given the same feed. The values are calories retained in the body per gram feed given in addition to a maintenance ration, and are measurements of the partial efficiency of feed energy utilization by the species concerned. The table shows that differences between species in the partial efficiency of feed utilization are not constant but vary with the character of the feed given. With cereals, pigs and hens have a partial efficiency 50% greater than that of ruminants, and the hen is more efficient in this regard than the pig. With wheat bran of high extraction the pig is superior to the ruminant but the hen is not, and with still more fibrous materials the ruminant excels by a factor of over 2. The generalization can be made that the hen excels in dealing with highly digestible cereals, the pig in dealing with by-products of human food preparation and the ruminant in converting to edible product the fibrous grasses, legumes and straws of its feral diet.

The partial efficiency of protein utilization is as a first approximation its biological value if protein intake is measured in terms of digestible protein. When comparisons are made between pigs and poultry given the same type of ration, the fact that their amino acid requirements differ radically makes considerable difference to the results obtained. The chicken requires glycine whereas the pig does not, while the chicken requires considerably more arginine and phenylalanine than does the pig. This means that particular diets though adequate for pigs are not adequate for poultry, a very clear example being experimental diets in which casein is the sole source of protein (44). In general, however, modern rations give approximate equal partial efficiencies of protein utilization in pigs and poultry since feeds are mixed together to meet particular amino acid needs.

TABLE 6. *Comparison of Species With Respect to Net Energy Values of Different Feeds*

Feed	Absolute Values kcal Energy Gained/g D.M.			Relative Values		
	Ox	Pig	Hen	Ox	Pig	Hen
Maize grain	2.04	3.01	3.05	100	147	150
Earthnut cake	1.77	2.61	2.28	100	147	129
Wheat bran	1.50	1.71	1.06	100	114	71
Alkali straw	1.97	2.02		100	102	
Dried grass, 13% protein	1.83		0.59	100		32
Wheat chaff	0.63	0.28		100	44	0

Compiled or calculated from the results of Fingerling (24–26). Kellner (38), Deighton and Hutchinson (21), Werner and Franke (62), Heinzl (35), Fraps and Carlyle (29) and Armsby (1).

The ruminant animal is virtually independent of an exogenous source of amino acids provided a source of nitrogen and sulphur is given. This means that in the growing ruminant animal the biological value of dietary N × 6.25 departs little from a value of 65 and in the milking animal from one of 70. Ruminants thus have a contribution to make to human diets which far outweighs any inefficiency with which they may be credited. They can by virtue of the microflora of their guts synthesise protein of high biological value to man from sources which are either poorly utilized by nonruminants or cannot be utilized by them at all. The synthesis of B complex vitamins by the microflora of the ruminant gut and their subsequent secretion in milk may be regarded in much the same way.

MAINTENANCE NEEDS OF DIFFERENT SPECIES

The above discussion deals with partial efficiencies of feed utilization, that is with the increments of energy or protein retained per unit increment in feed given. The total efficiency of feed conversion is the total gain of the animal, whether expressed as crude weight or as calories divided by the total feed. It therefore includes the cost of maintaining the animal. Obviously, if feed is given in just sufficient amount to meet maintenance needs, then total efficiency is zero. If gain is directly proportional to

food intake above the maintenance level, that is partial efficiency is constant at all feeding levels, then total efficiency increases with increasing food intake. Any comparison of the efficiency of different species must, therefore, take into account species differences in maintenance, and it would seem that those species with high maintenance needs would be the least efficient. This, however, is not true if the voluntary intake of feed is proportional to maintenance needs. Kleiber (40) was the first to point out that the efficiency of an animal must be proportional to its relative feed level, that is, the ratio of its intake of feed to its maintenance need and that broadly speaking efficiency must be independent of body size (39). In confirmation of this generalization Guilbert and Loosli (32) showed that for all species of farm animal "the total nutrient intake is proportional to the same fractional power of body weight as the basal metabolism and the maintenance requirement." They further stated "if all species conformed exactly to the curve representing weight raised to the power 0.75, then one would expect all to be about equally efficient in converting feed nutrients into body substance." However, there is evidence from the data presented by Guilbert and Loosli that in early life pigs and poultry have a greater relative feed capacity than have ruminants and hence are the more efficient. It is only at maturity that Kleiber's generalization applies.

SPECIES DIFFERENCES IN APPETITE

The salient importance of relative feed capacity as a determinant of efficiency raises problems concerned with the factors which control the maximal food intake of different species. There is considerable evidence to show that appetite for food is determined by both the physical distention which food causes in the gut and by homeostatic mechanisms whereby the animal equates its intake of energy to its particular needs (10, 49). Thus when poultry are given diets differing in productive energy value the birds consume more of the diet with the lower energy value, thereby maintaining constant their energy intake. Many examples of this might be quoted both for growing birds (see for example 55) and for adult pullets and hens (see for example 15, 16). This same phenomenon of homeostatic regulation of calorie intake when feed is given ad libitum is seen in the pig as judged for example in the data assembled by Lucas and Calder (46).

Undoubtedly some stage is reached in both these species when the individual is physically incapable of increasing its feed intake to compensate for a decline in its nutritive value per unit bulk. For example, the '6-day chick disease' which occurred in the United Kingdom when very poor quality feed was given, was certainly due to starvation of the birds consequent upon their inability to eat sufficient of the 'dilute' rations.

With ruminant animals given fibrous materials such as hays and straws, the physical effects of food on appetite regulation predominate and there is a positive association between voluntary intake and the nutritive value of unit weight of ration (8). As the energy value of unit food increases so voluntary intake increases, and it appears that homeostatic regulation of feed intake comparable to that seen in poultry and pigs does not operate until feed is given with an apparent digestibility of over 80% (12). In other words, for practical purposes, improvement of the nutritive value of a ration for ruminants should result in a more spectacular improvement of total efficiency of feed conversion than it does in the nonruminants, simply because it enables the ruminant to attain a higher relative feed capacity. Conversely, the greater relative feed capacity of growing pigs and poultry compared with ruminants as pointed out above, may not reflect a real difference between species. Rather it can be interpreted to show that the ruminant animal's feed supply is usually of such low concentration that homeostatic regulation does not occur but that the rations of poultry and pigs result in no physical limitation of appetite.

SPECIES DIFFERENCES IN EFFICIENCY

From the above discussion comparisons of the energetic efficiency and of the efficiency of protein conversion by different species, even when not complicated by the economic considerations previously mentioned, are complicated by the difficulty of finding a common denominator which takes into account that species differ in the way in which they utilize feed and that such differences are not constant but vary from ration to ration. For example, to compare species on the basis of their dry feed intake is to penalise ruminants which consume feed of low digestibility; to compare on the basis of TDN determined with ruminants tends to penalise omnivora which digest feed better than do ruminants. To compare on the basis of species specific net energy values such as those given in table 6 is to remove almost entirely any species differences which might exist. In most of the detailed comparisons of the efficiency of feed utilization which have been made, such criticisms apply and have indeed been realised by some of the authors concerned. For example, Leitch and Godden (42) criticised the efficiencies calculated by Armsby and Moulton (2) because the latter employed the calorific value of the feed dry matter as the measure of the denominator and the calorific value of the gains as the measure of the numerator. On this basis Armsby and Moulton showed that fattening cattle had an efficiency of 10%, fattening pigs an efficiency of 31% and milking cows an efficiency of 17%. Leitch and Godden (42) carried out the computation using digestible energy as the denominator and yield of edible material as the numerator and found efficiencies of 16% for fattening cattle, 20% for milking cattle and 22% for fattening pigs. They in turn were criticised by Bolton (15) who rightly pointed out that the digestibility figures employed in their compilation were those for cattle, thereby penalizing poultry, which digest their preferred rations more efficiently than do cattle. Bolton (15, 16) showed that the energetic efficiency of

fowls producing 200 eggs per year was in fact 17.7%, that is very much higher than the value of 11.8% given by Leitch and Godden. Bolton (15) indeed states 'the fowl laying 200 eggs per year converts the food it digests to human food more efficiently than does the 600-gallon cow.' As a final example, Halnan (33) computed the efficiency of different species using as basis the net energy value of food determined with ruminants, and found that the energetic efficiencies were 48% for milk production, 39% for poultry meat, 37% for beef and about 45% for the pig.

It will be seen that these attempts to estimate the comparative efficiency of different species give results which can almost be predicated from the premises made about the basis for comparison. How then can species best be compared? Perhaps Sir Thomas Middleton's approach in 1923 (50) was valid. Middleton calculated the efficiency of food conversion in terms of the amount of human food as animal product produced from the crops and grass produced from an acre of land. The calculation applied to the United Kingdom and to land of high fertility and his conclusions were that the milking cow excelled. This approach is a valid one and in effect it introduces into the question "which species is the most efficient of our domesticated stock" the additional phrase, "in utilizing land, labour and other resources for human food production." In other words, species can be compared one with another only in the context of a particular set of resources and in a particular environment. Furthermore, in considering land usage direct consumption of crops by man has to be considered also, particularly in those countries where human food is scarce. When this is done consideration of the relative efficiency of different species as food convertors often becomes an academic question. To take an extreme example, some estimates of the efficiency of laying hens show they are more efficient than is the ewe producing a lamb. To replace ewes by poultry on Scottish hillsides which can barely support 1 ewe to every 9 acres is warranted by such calculations but is quite obviously ridiculous: apart from the nutritional considerations the hens would be blown away!

The planning of the agriculture of an area to support a human population obviously must take into account the efficiency of livestock as convertors of feed to human food. We should not be concerned, however, with the comparative merits of different classes of stock nor with the comparative merit of plants and animals in producing human food, but rather with their complementary merits in an integrated economy. Livestock provide a means for the utilization of land resources that cannot be used directly to produce human food, they can act as scavengers of waste products of the food industry, they can convert surpluses into more acceptable products, and they can concentrate, or in the instance of ruminants, synthesise vitamins and protein from low grade sources to provide supplements for food grains. In accomplishing this overall integration of meat, milk and egg production, the production of food grains, pulses and vegetables and of the production of hides, wool and vegetable fibres within the context of a complex set of natural resources, the final and only real criterion of efficiency is the health and well-being of man.

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Relation of nutrition of the young animal to subsequent fertility and lactation

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In 1947 THE SENIOR AUTHOR had the opportunity to visit the University of Missouri. On this occasion Dr. A. G. Hogan told about the work on animal nutrition at the University. He mentioned with great respect Dr. H. J. Waters and the pioneer work on nutrition in growing cattle, initiated by him in 1907.

In order to understand the importance of the Missouri work, it should be taken into account that a liberal and rather uniform nutrition without fluctuations at that time was considered necessary to secure 'normal' growth and development (54, 66, 93).

EFFECTS OF NUTRITION ON GROWTH

General considerations. Some years ago, Jackson (58), reviewed what was then known about the influence of inanition and malnutrition on growth, stressing the structural problems. His conclusions are still of great interest.

Among the living species, vertebrates seem the most resistant to inanition. However, the resistance may vary according to species, age, sex, and individual. Among the factors influencing the resistance mention may be made of the reserve material of the body. The nervous system and the skeleton are the most resistant, the adipose tissues the least.

According to Jackson, "the cells during inanition pass through two stages." The 1st stage is a simple atrophy leading to a more embryonal condition. Sooner or later this stage passes into a 2nd stage, characterized by degeneration. The whole cell may then disintegrate and disappear.

It is important to note that in the atrophic stage, the cells recover rapidly. In case of extensive degeneration, the ability of regeneration is lost, at least partially, as there still may be some cells which are capable of recovering. This means that "up to a certain stage of severity, perfect recovery is possible" and "beyond this stage only partial recovery is possible" (58). There are critical periods, especially at an early age, during which there is danger of not only depressed, but also of abnormal growth.

Jackson discussed various theories that may account for the differences in resistance between cells and organs. It may here be sufficient to mention that, as was to be expected, the organs most important to the animal seem to be the most resistant ones.

Hammond and his school (McMeekan, Wallace, Palsson, Verge) have by the aid of the dissection method greatly extended the knowledge of growth, and its relation to nutrition. Hammond's theory of differential, or heterogenic, growth deals with the general principles of growth and development in farm animals (81). Various organs and tissues differ as to the maximum rate of growth. Relatively, the order of development is as follows: *1)* brain and central nervous system; *2)* bone; *3)* muscle; *4)* fat. The partition of the supplied nutrients on the different tissues is determined by the order of development (44). In cases of undernutrition, the conformation may be influenced. Whether there will be any lasting effects depends on the severity of the undernutrition (81).

When discussing the extent to which growth is unfavourably influenced by partial inanition, it seems appropriate to distinguish between essential nutrients and energy, at times referred to as the quality and the quantity of the diet.

If one or another essential nutrient is removed from the diet, or kept at a very low level, the result will be deficiency diseases, specific to each nutrient. It is beyond the scope of the present review to discuss deficiency diseases in general. In connection with our topic, the main point is to what extent animals may stand partial deficiencies, and whether they are capable of repairing the damages once they have developed. Three important factors must here be taken into account:

Age. At an early age, the period of the most intensive growth, the animal is more susceptible to irrepairable damages from deficiencies and from undernutrition in general.

Severity of underfeeding. As to some of the essential nutrients, the animals have at their disposal body reserves, that may be drawn upon, for instance calcium, phosphorus, and fat-soluble vitamins. As far as some other nutrients are concerned, the body reserves are depleted rather quickly, after which deficiency symptoms develop. The animal may to some extent protect itself

by reducing its feed intake. The possibilities of restricting the intakes of essential nutrients will be limited. That explains the interest in stating the minimum requirements, which prevent observable deficiencies, while optimum requirements sometimes called allowances, include a margin of safety which in some cases are none too well defined.

Duration of period of underfeeding. It goes without saying that the risk of deficiency damages increases with length of time during which the animals are exposed to underfeeding.

It is a matter of dispute whether stress should be laid on daily intakes or percentage levels in balancing feed rations with protein and other essential nutrients (7, 24, 25, 71, 101).

At the present state of knowledge of nutrition feed rations can easily be balanced as to prevent gross deficiencies. The progressive farmer of today, therefore, need not be too much worried in this respect. The problem as to what extent animals can stand variations in feed intakes during growth, however, is important economically. Energy and protein being quantitatively the decisive factors in nutritive requirements, it follows that variations, first in energy intakes, and secondly in protein intakes, are in the foreground when effects on growth are discussed. In the broiler nutrition relative costs of supplying the main groups of nutrients have been calculated at: energy 60%, protein 33%, minerals 4%, vitamins 3% (8). These figures illustrate the importance of energy and protein from an economic point of view.

In the case of balanced rations and healthy animals the growth rate seems to be determined mainly by energy intake, as shown by Crampton (26) in pigs. By supplying energy in relation to age it is possible to keep animals growing in accordance with standard weight curves, as shown in our laboratory with pigs (55, 101), and by Brolund Larsen (18) in calves.

EXPERIMENTS ON GROWTH IN BEEF CATTLE

In the above-mentioned pioneer studies carried out at Missouri, the 'Use of Food Experiment,' Waters, in 1908, demonstrated that calves continued to grow when kept on a maintenance ration, that did not allow any gain in weight for several months. The height at withers proved a good measure of skeletal growth. A total of 59 animals were used in the experiment (72–74). At the age of 2–7 months, the majority of the animals were divided into 3 groups, fed in dry lots in the following way:

Group I, full feeding, permitting maximum fattening
Group II, feeding for maximum growth, without surplus fat storage
Group III, feeding for retarded growth

In addition to the milk fed during the early growth period, all groups were fed grain and hay, in the ratio of 2:1.

For the animals fed through a period of 4 years, the following figures may serve as an illustration of the results attained:

Group	1	2	3
Daily gain, lb.	1.30	0.80	0.65
Dry matter per lb. of gain, lb.	12.24	11.91	11.03
Height at withers	140–150 cm in all groups		
Weight, lb.	1965	1255	1046

While the animals in *groups II* and *III* at 4 years had reached the same height as those in *group I*, there was a wide difference between the groups in body weight, and in measures of length, width, and circumference of the body. Thus, in some respects, it was not possible to regain that lost at the low level feeding, although longer periods of liberal feeding prior to slaughtering might have brought about recovery in a greater measure. The difference between the groups mainly was due to a difference in body fat, as shown by the following figures for animals at 3½–4 years of age (73, 74).

	Fatty Tissues, %	Fat in Total Animal (Empty Weight Basis) %
Group I	about 40	about 45
Group II	about 19	about 20
Group III	about 11	about 10

The 'Use of Food Experiment' was followed by a special investigation on the permanency of the effects of retarded growth (54, 87). Fifteen animals were divided into 3 groups, and fed different planes of nutrition:

Group I, normal growth, 1 lb. of gain a day
Group II, retarded growth ½ lb. of gain a day
Group III, retarded growth ⅛ lb. of gain a day

The period of underfeeding in *groups II* and *III* ranged from 3¼ to 6½ years. After refeeding (full feeding) for a varying time, some animals attained normal size, while others did not. The underweight of the latter, however, did not exceed 10%. In the underfed animal there was a considerable prolongation of the growth period. The underfeeding did not affect the body measurements, concerning the skeletal growth (54). According to other investigators, underfeeding may, however, influence the measures concerning body conformation (46, 57, 81). It was indicated that about 7% of fat in the body (empty weight basis) is the minimum fat percentage compatible with survival (87).

Studies similar to these conducted at Missouri have been carried out with identical twins at Beltsville (105, 106). One animal from each pair was underfed to a varying degree, the other serving as control. Some animals received a maintenance ration for 6 months, between 6–12 months of age. Others received only ¾ of a maintenance ration for 3 or 4 months, starting from 3

or 4 months of age. The control animals and the experimental animals after the termination of the periods of underfeeding received a liberal feeding. At all feeding levels liberal quantities of the essential nutrients were fed. All animals were fed in dry lots.

The only effect of the rather severe underfeeding in these well planned experiments, was a prolongation of the total growth period. In other respects, the recovery after the underfeeding periods was nearly perfect. The experimental animals attained the same end weights as did the control animals. There were no differences in body measures, even those concerning widths, nor in the fat content of the rib cut, or in the meat quality. It is remarkable that the experimental animals, in spite of the prolongation of the growth periods, produced the body gain nearly as economically as did the control animals:

	lb. TDN per lb. of Gain	
	Control	Experimental
Experiment 1	5.8	6.0
Experiment 2	6.0	6.1

After the periods of underfeeding, the growth in the retarded animals was more rapid than that in the controls, thus partly compensating for the retardation, a possibility indicated already by Waters (104).

According to these experiments, a quantitative restriction in the feed, for a limited time, may be used with advantage in beef production when feed supplies do not allow continuous growth.

How economic considerations may influence the choice of feeding levels in beef production have been aptly demonstrated by Brookes and Vincent (19). A high plane of nutrition the first 8 months, followed by a moderate level, was most economic, because of the use of cheap, bulky feeds during the latter period.

EXPERIMENTS WITH DAIRY CATTLE

Females. At Missouri University, a pioneer study was carried out by Eckles, over a period of 8 years, beginning in 1906 (34, 35). Forty animals were fed 2 different diets. One group received whole milk the first 6 months, and as much grain and alfalfa hay as the animals could consume up to the first calving. Another group received skim milk for 6 months besides alfalfa hay, but no grain until the first calving. After calving, both groups were fed the same rations, in proportion to the milk production. Some animals in each group had access to pasture, or green feeds. Two different calving ages were used, 20–24 months and 28–34 months.

As might have been expected, the extreme restriction in the roughage group caused some depression in growth, in comparison with the heavy-fed group. At the age of 5 years, there were still differences in size between the groups.

The gestation only slightly influenced growth, while lactation greatly suppressed, not only growth, but the final size as well, especially, when early calving and light feeding were combined.

Pasture partly compensated for the light feeding indoors, in respect to live weight gain.

The first estrus was noted at an average age of 9 months in heavy-fed heifers, and at 12 months in light-fed heifers.

The heavy-fed animals had somewhat higher yields during the first lactation than the light-fed ones. In the 2nd lactation period, there was no difference in yield between light- and heavy-fed animals of the Holstein and Ayrshire breeds. In the Jersey breed, the yield for the 2nd lactation period was even higher in the light-fed than in the heavy-fed animals, 285 against 228 lb. of butter fat. The results pointed to 24–30 months as a desirable calving age.

At Kansas University, (83) heifers were fed alfalfa hay exclusively, from 6 months of age, not only up to the 1st calving, but to the end of the 2nd lactation. Since hay rations cannot supply the energy needed in milk production (56, 88) it is understandable that the milk yields at this feeding regimen were lower than in cows fed also silage and grain.

One may not be wrong in guessing that the Kansas studies were inspired by the Missouri-studies. The same possibility appears in connection with the comprehensive Danish experiments on nutrition in growing cattle (37, 47, 93–95). These experiments were initiated by Professor Lars Frederiksen, Head of the Division of Cattle Feeding and Breeding at the Danish State Research Laboratory, from 1923 until his untimely death in 1933. Frederiksen studied in the United States in 1912–1913, and while there became acquainted with Eckles and his work. Frederiksen's co-worker, Dr. V. Steensberg was in charge of the Danish experiments with growing cattle, from the start of these in 1928 until 1949, when he left the laboratory.

The great extent of the experiments is shown by the number of animals, carried through to the first calving:

	Duration of Experiments	Group B	Group A	Group C
Red Danish	1928–37	58	62	51
Black and White Jutland	1931–41	24	25	25
Shorthorn	1931–41	25	26	25
Jersey	1948–56	55	49	50
Total		162	162	151

The normal plane of nutrition, used in *group A*, was based on physiological considerations (41, 94). It was shown that the production feed could be related to the expected final weight of mature animals. At the low-feeding level (*group B*) the total indoors feed intake, measured in feed units, was 25–30% below the normal level, while at the high-feeding level (*group C*) the feed

intake was 25–30% above normal. During the summertime, all groups had access to the same pastures. As in Eckles experiments, the effects of the low-level feeding indoors were partly compensated by the pasture feeding, as shown in the following figures for live weight gain:

Groups	B	A	C
kg of gain indoors	180	244	282
kg of gain on pasture	146	114	110

At the first calving, some differences in weight and body measurements between groups were found. Then all groups were placed on the same feeding level, and after the third calving, the difference in average size in Red Danish and Black and White, was negligible, while in the early maturing Shorthorn, the difference between *group B* and the other groups still persisted. The influence of the calving age on the weights is shown below (95):

Groups	B_I	B_{II}	A
Age at first calving, years	2¾	2⅛	2⅛
Weight after first calving, kg	437	371	415

Information about the effects of the varying feeding levels during the growth period on milk yields, fertility, and longevity, in the Red Danish and the Jersey breeds are available (37, 47). The milk yield measured in kilograms of F.C.M. was:

Breed		Group B	Group A	Group C
Red Danish	1st lactation period	3235	2922	3469
	2nd lactation period	3892	3563	3869
Jersey	1st lactation period	3739	3580	3406
	2nd lactation period	3940	3667	3638

Thus milk yield is not lowered by light feeding during the growth period. In the Jersey, it even seems that such a feeding regimen favourably influences the milk yield. As mentioned above Eckles attained similar results in this breed.

In the light-fed animals, the fertility was somewhat higher than in the heavy-fed ones. Excluding tuberculosis and low yields, the discarding in Red Danish because of infertility and returning was as follows:

Group	Uncorrected	Corrected (for Contagious Abortion)
B	42%	38%
A	54%	46%
C	72%	50%

As might have been expected from these figures, the productive life space was shorter in the heavy-fed animals (*group C*) than in the other 2 groups. The average number of calvings per cow was: *group B*, 4.6; *group A*, 4.3, *group C*, 3.4.

While 65–68% of the cows in *groups A* and *B* had more than 3 calvings, the corresponding figure in *group C* was 35% only. The difference in endurance between the light-fed and the normally fed animals is negligible.

In the Jersey also the heavy feeding during growth seems to have influenced fertility and health unfavourably.

In New Zealand, extensive investigations on different levels of nutrition during growth in cattle, and their effects on the lifetime production, have been carried out (2, 3). One project, covering the period of 1944–1957, comprised a total of 301 animals of the Jersey breed, at the age of 20 months. As usual in New Zealand, the feeding of the cattle was mainly based on pasture. One group was given a high level of nutrition by means of rotational grazing, autumn sawing of pasture, and maximum use of hay and silage. Another group received a low feeding, characterized by great variations in feed intake, caused by the seasonal fluctuations in grass growth. At the first calving the animals were divided into 4 groups, which were put on the feeding planes described above:

Before first calving	After first calving
High	High
Low	High
High	Low
Low	Low

The average weight of well-reared animals at the age of 20 months was 724 lb. to only 579 lb. in the poorly reared group. At the first lactation, the well-fed animals (high-high) had the highest yields. The yields for all lactation periods, 1947–1957, were as follows:

	No. of Lactations	Lb. of Milk Fat
High-high	242	347
Low-high	235	345
High-low	233	308
Low-low	234	305

According to this large amount of material, poor feeding during growth does not depress milk yields in the 2nd or following lactation periods.

In the preliminary reports available at the moment no information is given about fertility and longevity.

In the Northeastern region of the U. S. A. a cooperative study entitled *Causes and Prevention of Reproductive Failures in Dairy Cattle* (N.E.-1) has been in progress for some years. At Cornell University the effects of various levels of nutrition during growth has been studied in Holstein cattle (84). The following 3 levels of nutrition have been used during the growth period:

Low	65% of T.D.N. prescribed in the Morrison feeding standard
Medium	100% of T.D.N. prescribed in the Morrison feeding standard
High	140% of T.D.N. prescribed in the Morrison feeding standard

The animals on the low-level feeding were offered poor pastures. The other groups grazed good pastures, the high-level group even being supplemented with

concentrates. During the first lactation period the low-level groups were given more feed than the high-level group. Later, all groups were fed alike.

The Cornell experiments are still in progress, and a cautious interpretation of the preliminary data is therefore advisable (86).

At the 1st calving there was a great difference in size between groups, but already at the 2nd calving the difference in body weight between the low and medium groups was levelled.

As to the milk yield there was no significant difference between groups up to 1957, although it seems justified to point to a slight tendency towards lower yields in the high-level group. The cumulative T.D.N. intake and milk yields are given below (86):

Feeding Level	No. of Cows	Lb. F.C.M. During First 4 Lactations	T.D.N., lb (as Barn Feeds)
Low	13	40,959	27,366
Medium	18	40,627	28,577
High	14	38,397	31,157

The age at the 1st estrus in the low-, medium- and high-level groups was about 20, 11 and 9 months, respectively. At the time of the 1st estrus the animals in the different groups were of about the same size, as measured by height at withers, heartgirth, and body length. Some calving difficulties were noted in the low-level heifers at the first calving, but the animals conceived as well as those in the other groups later on (86). A few more animals were discarded for sterility in the high-level group than in the other 2 groups.

In another Cornell study (92) with similar feeding levels, and dealing with the development of reproductive and endocrine organs, the ages recorded at the 1st estrus were approximately the same as those mentioned above. The rate of development of the reproductive organs was similar to that of the estrus.

Once the estrous cycles had begun, they occurred with about equal regularity in all 3 groups. It is considered advisable to breed heifers relatively soon after they start cycling. Heavy feeding increased thyroid activity by stimulation through the pituitary. It is indicated that the increased metabolic rate resulting from these changes, and expressing itself also in increased heart and respiration rates, may shorten the life span.

In a Missouri study (53) depressed milk yields and fat deposition in the udder before calving were noted in heavy-fed heifers. It is recommended to feed liberal roughage quantities with minimum grain after the age of 8–9 months.

At Wiad, Sweden, comprehensive experiments on the effects of different levels of nutrition in cattle during growth have been carried out since 1941 (11, 12, 51, 52).

Identical twins of the S.R.B. (Swedish Red and White Cattle) breed were used. Identical twins are well suited for growth studies, and the pioneer work at Wiad has animated many research institutes to use twins. Four experiments have been carried out at Wiad. They comprise the following number of twin pairs:

Experiment	Started	No. of Twin Pairs
I	1941	9
II	1943	8
III	1947	16
IV	1950	27

The space does not allow any details regarding the design of the experiments. It may suffice to mention that in *experiments I* and *II*, a low-level feeding was compared with a high-level feeding, up to the first calving. In *experiments III* and *IV*, 1 twin from each pair was kept as a control, on a normal feeding level, according to standards, the other receiving varying feeding levels, 40, 60, 80, 120, and 140% of standard. The feed quantities actually consumed ranged from 51 to 124%. The feed intakes in the 2 experiments were recorded up to an age of 25 months. The information about the feeding during the interval between this age and the 1st calving is somewhat scarce, but it is important to note that in these 2 experiments all animals seem to have received a rather liberal feeding during the period preceeding the calving. After calving, all animals were fed according to the same plan. In all experiments dry lot feeding was used.

As in the experiments mentioned above, the different feeding levels greatly influenced the weight of the animals at the age of 2–3 years.

In the 2 first experiments, the milk yield, measured in F.C.M., was about the same in the high- and the low-level animals. The milk yield in the 2 later experiments were as follows (51):

Planned Level of Nutrition	F.C.M., kg	
	First lactation	All lactations
60	3117	3328
80	3145	3244
100	3016	3119
120	2866	2908
140	2573	2635

Heavy feeding during growth thus seems to depress the milk yield. The 60–80% level was actually shown to give the best lactation results, when combined with a preparatory feeding, before the 1st calving. It is indicated by Hansson that fattening during growth depresses the lactation ability.

High feeding during growth tended to increase the milk-fat percentage in the first lactation period. The 40 (51)-% level greatly retarded the development of the reproductive organs, and was not considered practical.

A relationship between feeding level and heart and respiration rates was demonstrated, as in the Cornell studies mentioned above. In *experiments I* and *II*, excluding animals discarded for low yields, the life span for

the remaining 9 pairs averaged 7.9 years for light-fed animals to 6.3 years for heavy-fed animals.

The advisability of combining light feeding during growth with a kind of preparatory feeding prior to calving, has been indicated in experiments by Flux (39). Of 6 pairs of identical twin heifers, 1 in each pair was underfed on a poor pasture, for a period of 10 months, while the pairmate received a liberal feeding on good pasture, hay, and silage. The average milk yield during a period of 270 days, was 3187 and 4244 lb., respectively, i.e. a difference of 25%.

In mature cows, and occasionally even in heifers in good condition, preparatory feeding before calving does not seem to have any beneficial effects (42, 61, 89). Careful Danish studies conducted by Jakobsen (59) have demonstrated that it is important to supply liberal amounts of protein the last month before calving. This bears out the view that in balancing feed rations not only the percentage levels, but the daily intakes as well should be taken into account.

In our laboratory we have the experience that heavy, longlasting preparatory feeding is not to be recommended (15). A 'transitory' feeding, a moderate increase in the feed level 2 weeks before calving, combined with a gradual increase from the 3rd day after calving, was recommended (14). It seems to correspond fairly well with the so-called 'steaming up,' which has been used in some experiments in England with good results (9, 10, 20, 21). It is easier to make the animals consume grain immediately after calving, when they have received some grain prepartum (67).

At Rowett Research Institute, a series of experiments on different planes of nutrition during growth have been carried out (27, 28). Twenty-four pairs of twins of Friesian and Ayrshire breeds were divided into 4 groups, fed according to the plan below:

Group	First 10 Months	Until 2 Months Before Calving	Last 2 Months Before Calving
HH	High	High	High
HL	High	Low	High
LL	Low	Low	High
LH	Low	High	High

The high plane was designed to be 110% of the Ragsdale standard (82), and the low plane 70% of this standard until the animals were 6 months old, and 60% later on. Having reached an age of 10 months, the high-plane animals were placed on good pastures (leys) during the summertime while the low-plane animals grazed rough hill pastures. The last 2 months before calving, all animals were fed on a rising plane of nutrition. After calving they were fed according to standards with the same maintenance feed for all groups.

When the animals were 24 months, there was a considerable difference between groups in body weight and in body measurements, even height at withers. However, for animals of 5–6 years, the body size was approximately the same in all groups. Thus, no permanent stunting occurred, but maturity was delayed about 9 months in the *LL group*, and 4–5 months in the *LH* and *HL groups*.

The first estrus occurred at different ages, but at about the same size, as shown below:

Group	Age in Months	Body Weight, lb.	Height at Withers, in.
HH	12	565	43·7
HL	18	548	44·1
LL	15½	525	43.6
LH	14½	567	44·3

The changeover from a high to a low plane in the *HL group* had an adverse effect.

Results as to milk production and productivity have not as yet been published (April 1960). According to a personal communication, the differences in milk production between groups are rather small. In preliminary reports the *HL group* was stated to be lower than the other groups (21).

In Eastern Germany, experiments on different feeding levels in growing cattle are in progress (75). Immediately before the first calving, the difference in body weights between the 60 and 100% feeding levels, was 116 kg at early calving and 74 kg at late calving.

An interesting observation on fertility in cows was made in Eastern Germany by Stahl. Cows calving in January–February had longer calving intervals than those calving in any other month of the year. This was ascribed to difficulties in conceiving, owing to shortage in feed supplies at the end of the indoor feeding period. This finding may be in line with the decrease in conception rates in cows during April–June as demonstrated by Edwards (36).

In South-Africa (64, 65), 2 groups of heifers were raised on a low and a high plane of nutrition. The animals on the low plane were grazed throughout the year up to calving, while those on the high plane, were given supplementary feeding during winter (May-October). The sexual activity of the heifers on the low plane was strongly depressed during the wintertime, and not restored until the weight losses had been regained.

In these studies, comprising 28 animals, the influence on nutrition on growth and milk production has been studied. The poor winter feeding for the low-plane animals retarded the growth. Even after 4 years, differences in size were evident. It is interesting to observe that the stunting was most severe in the Shorthorn breed, while the Jersey breed was least affected. These results are in good agreement with those obtained in the Danish studies mentioned previously. The milk production, given for 14 animals, was as high in animals reared on a low plane as in those reared on a high plane.

At the University of Tennessee Swansson (96) carried out experiments with 7 pairs of identical twins, in which

the Jersey breed was predominant. One animal in each pair was fattened by being fed concentrates according to appetite, while the other received an ordinary feeding level. During the first lactation period, the average milk yield in the fattened animals was only 85% of that in the normally fed animals. The udders of the fattened animals were incompletely developed as to the alvuolar system. It was thought, however, that there might be still other causes of the poor lactation ability in the fattened animals. The results from the Tennessee studies are in line with those from Sweden and from Missouri, and from the Danish studies in Jersey. As in South-African studies heavy-fed heifers lost considerably more weight after calving, than did the light-fed ones.

In comprehensive experiments at Beltsville (99) heifers were reared on different roughage rations, alfalfa hay, and silage. The nutrient intake was lower for the animals on silage than for those on hay. The animals reared on silage, were at 2 years, smaller, and the milk production during the 1st lactation period tended to be lower, than in animals reared on hay rations. At the age of 4–6 years, there were only small differences in body weights. The reproduction performance was not influenced by the rearing rations.

As to longevity and fertility, it has been maintained that individual differences are largely determined by nongenetic influences (80). Various aspects of the relation of fertility to nutrition, have been discussed (5, 36, 40, 45).

It has not been proved that a liberal feeding after the conclusion of the growth period has any detrimental effects on fertility and health in dairy cows (48, 62).

Males. A low-feeding level for raising bulls has in several experiments led to delayed growth, delayed sexual maturity, and in some cases, to a smaller volume of ejaculate, lower sperm concentration, and sperm mobility (1, 13, 31, 38, 60, 77). In a Swedish study (6) a similar effect of nutrition on growth was shown. The serving behaviour, however, was mainly determined by inheritance. Most of the above-mentioned experiments were carried out with identical twins. The lowest-feed level used was 60–70% and the highest level about 150% of the common standard. As to the other results from these studies, it should be mentioned that an excessive use of bulls as yearlings reduced their subsequent sexual performance, which, however, did not seem to have been influenced by exercise and season (1).

The experiments seem to confirm the statement made by Walton (103), that the needs in the semen production will be met by a nutrition adequate to maintain body health.

OTHER SPECIES

The present review is based mainly on existing experimental evidence for cattle. This seems justified as far as the economic importance of this species is concerned.

In order to discuss whether the results attained in cattle have a general bearing on nutrition, we shall shortly mention some results from growth studies in other species.

In experiments with sheep at Cambridge (81), a severe underfeeding during the last 2 months of pregnancy, followed by a low level of nutrition after birth, depressed the growth to an extreme extent, and, also affected the carcass proportions. As in the case of cattle (57, 81) the body measurements relating to the width were most depressed by undernutrition. Severely underfed animals, therefore, will be large, but narrow and thin. It may be of interest to note that external body measurements indicate the length growth of the skeletal parts, and may, therefore, be somewhat inadequate in describing the actual growth as stated by Palsson (81). Underfeeding during the last part of the gestation period, has been shown to depress birth weights of the lambs and udder growth and milk production in ewes, thereby also affecting the growth and survival rate of the lambs (29, 46, 81, 100, 102).

In a study conducted at Rowett (29) a low level of nutrition in 10–18-month-old ewes including their first pregnancy, was found to cause permanent stunting. Even when the animals were 6 years old, a high age in sheep, the weights were lower than those of the controls. Since sheep commonly carry twins, the gestation period creates great demands in this species. This may explain why undernutrition seems to affect sheep more than cattle. In New Zealand studies (cit. 85), however, a moderate underfeeding during growth did not affect the milk production in sheep. A high plane of nutrition during the mating season increases fertility (85).

The well-known Cambridge studies carried out by McMeekan (70) has shown that in pigs nutrition may affect body conformation and carcass quality. Studies in Denmark and Minnesota (49, 63) have shown that a very rapid growth during the preweaning period has affected the growth and health during the period up to slaughtering adversely. In the sow, a high plane of nutrition, prior to mating, increases ovulation, while a low level after mating increases the embryonic survival, as shown in Wisconsin studies (cit. 85, 90). A restricted feeding during growth and gestation in gilts, may have a beneficial effect on the litter size (43, 50, 91). An increase of 1 mm in the backfat thickness during gestation decreased the litter size by 0.15 pig (32). In Danish studies a gradual increase in the feeding levels after farrowing has proved beneficial (23). In Rowett studies the feeding levels used for sows during lactation have not affected the fertility (30).

In experiments with dogs and rats Aron (4) early demonstrated that a prolonged underfeeding during growth may lead to permanent damages (78). The same was found by Jackson (58). Osborne and Mendel (78), on the other hand, found depressed growth in rats fed rations inadequate in quality or quantity, but on refeeding, the animals attained normal size by prolonging their growth period. It has been demonstrated that feeding ad libitum during growth is detrimental to the lactation ability in rats as is also the case in cows

(97). In Beltsville extensive studies on rats fed ad libitum and on rats fed a level 30% below that, from weaning to partition, were compared. The restricted feeding reduced the growth of the mammary gland, on being fed ad libitum during lactation, however, the restricted animals were superior, as to lactation performance (98).

The influence of the nutrition levels on the longevity of the animals has been a matter of great interest. In *Drosophila*, a prolongation of the larval period by using low levels of nutrients, increased the total life span from 19 to 29 days, i.e. by 53% (76). In 1917, Osborne *et al.* (79) reported a delayment of estrus and a prolongation of the life span in rats, stunted as a result of a low level of nutrition. Also in mice, reared on low levels of energy, a prolongation of life was observed (cit. 85). In the well-known Cornell studies (68, 69) restriction of energy intakes retarded growth, involving permanent stunting, but the life span was prolonged. According to Brody (16) the equivalent ages in humans would be 51 to 80–90 years. Rapid growth may thus reduce life expectancy (17). Intermittent fasting has been found to prolong life spans in rats by 15–20%, at the optimum amount of fasting, which appeared to be 1 day in 3 (22).

Even though some restriction of the diet during growth, seems desirable, a poor diet may have adverse effects, as indicated in Rowett studies with rats (29).

SUMMARY

The effects of different planes of nutrition in cattle during growth have been studied extensively in this century. By using feed rations, balanced as to essential nutrients, the energy intake has been varied to a considerable extent. Young, growing cattle have been kept at, or even below, maintenance level for several months, or at energy levels 20–40% lower than those prescribed in current standards for normal feeding, during the first 2–3 years of life. Alternating low and high planes have also been used. Restrictions in the energy intake have a retarding effect on the growth. By prolongating their growth period and growing more rapidly in the periods of liberal feeding following those of undernutrition, the animals are capable of recovering and may eventually attain the size of well-fed animals. A long-lasting, severe undernutrition at an early age, however, may lead to permanent stunting. Since the width measurements seem to be more seriously affected than the height measurements severe undernutrition may influence body conformation more than it does size. Breed differences as to the effects of undernutrition have been demonstrated. The Jersey has great resistance, while in the Shorthorn permanent stunting may occur, even at moderate restrictions in feed intakes.

In dairy heifers, energy intakes 20–30% below the current feeding standards during the 2–2½ years of early life, have no detrimental effects on the lactation ability. During the 1st lactation period, the milk yield may be somewhat lower than in well-fed animals, but during the subsequent lactation periods the animals reared on low planes of nutrition give yields at least as high as those of animals reared on the energy levels prescribed in the standards. It is then presupposed that after the first calving, the energy levels are equalized according to feeding standards. An increase in the feeding level, some weeks before the first calving, counteracts the lowering effect on the yield in the first lactation period, caused by low energy levels used earlier in life. Energy intakes exceeding those prescribed in the standards, for the rearing period up to first calving, seem to depress the lactation performance. This effect is more likely to occur in the Jersey than in other breeds.

It has been shown that the plane of nutrition influences the sexual development in cattle. Heavy-fed heifers attain the first estrus at an early age, while the estrus is retarded in animals reared on low planes of nutrition. The possibility of early breeding in heavy-fed animals can not be regarded as an advantage, as the fertility and longevity seem to be impaired by heavy feeding early in life. The experimental evidence available invites to some reduction in the energy levels prescribed in the current feeding standards for growing cattle in order to arrive at a more economic feeding. However, progressive stockmen, interested in the appearance of their animals are, however, not inclined to use low feeding levels.

Studies in other species confirm the results attained with cattle. When heavy feeding in cattle seems to be more questionable during early life than at a mature age, it must be taken into account that lactating cows have a great need for energy. In relation to their requirements, therefore, heavy-fed cows may receive less energy than heavy-fed young cattle.

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Recognition and correction of deficiency states

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LOCALISED AREAS IN many parts of the world have long been known to be unsatisfactory for the raising of stock. Such areas, in which endemic disorders of livestock occur as a result of qualitative abnormalities of the natural herbage, have now been recognised in every continent and more are being discovered and will be discovered as our understanding of the full nutritional requirements of animals grows; as the technical facilities for the detection of naturally-occurring departures from these requirements improve; as animal husbandry practices intensify in many parts of the world; and as the genetic and infectious disease factors limiting productivity per animal are increasingly recognised and overcome.

No worthwhile estimate of the total extent of endemic nutritional disorders of livestock in the world can yet be given, although it is clear that millions of square miles of the earth's surface are affected, but if developments in the last few years can be used as a basis for prediction, we are only at the beginning of an appreciation of their impact on food production. Within the last decade alone, extensive cobalt-deficiency areas affecting cattle raising have been delineated in Brazil, widespread deficiencies of iodine, copper and cobalt, and excesses of molybdenum and selenium have been demonstrated in the USSR (11), and indications have been obtained that selenium deficiency, or at least responses to selenium supplements, may play as important a part in the health and productivity of New Zealand livestock as does cobalt or copper (1).

In this paper an attempt is made to assess the general principles which underly the incidence, nature, causes, and means of detection and control of the various naturally-occuring nutritional disabilities which afflict the world's stock, rather than to present detailed descriptions of individual nutritional abnormalities. For these, reference should be made to the book by Underwood (17) and the extensive review by Russell and Duncan (16).

DEFICIENCY AND TOXICITY STATES

In the earlier studies of endemic nutritional maladies, attention was heavily focussed on the acute disease conditions which have well-marked and characteristic symptoms and high mortality. This was understandable in view of their drastic nature, obvious economic importance and often dramatic response to treatment. The recognition and correction of the milder nutritional disabilities, which have no clearly discernible symptoms and are usually characterised only by a vague unthriftiness, can be even more significant as it is becoming increasingly evident that they are more widespread and affect more animals and more acres than do the acute disorders. In other words, they take a much greater overall toll from prodution.

Mild, qualitative deficiencies or excesses are difficult to detect and control, particularly as their effects on the animal are often indistinguishable from those resulting from a primary energy-deficit, i.e. from starvation or semistarvation due to such factors as over-grazing or unpalatability of the herbage. This is because a depression in appetite is a common, although not an invariable, concomitant of individual nutrient deficiencies or excesses. Such a situation is well exemplified in the case of cobalt deficiency of sheep and cattle, which has no symptoms that can be regarded as specific for this element. The appearance of a severely cobalt-deficient animal is one of extreme emaciation and listlessness, indistinguishable from that of a starved animal, except that the visible mucous membranes are blanched. In areas marginal in cobalt status the animals exhibit only a mild, ill-defined, and often transient state of unthriftiness which can only be diagnosed with certainty as cobalt deficiency by the response which follows from dosing with cobalt or injecting vitamin B_{12}.

A further difficulty arises from the fact that deficiency and toxicity states which appear clinically, pathologically and even biochemically identical or similar in the animal may develop as an end result of different causal circumstances. This fact has given rise to a number of apparent anomalies in studies of several endemic disorders of livestock, some of which are not yet resolved, although there seems little doubt that they are related to an imbalance of nutrients. Simple or uncomplicated deficiency or toxicity states in animals are, in fact, comparatively rare under natural conditions. More usually they are ameliorated or accentuated, i.e. 'conditioned,' by other dietary factors which vary in different environments. These conditioning factors, as the following examples indicate, can so influence the metabolism of other nutrients that they largely or completely determine whether or not a particular deficiency or toxicity state will occur in a given environment.

In parts of Australia, for instance, chronic copper

poisoning in sheep occurs under 3 different sets of conditions with a similar clinical picture in the animal in each case. In 1 area the copper poisoning arises as a straightforward consequence of excess gross copper intakes from the herbage; in another area the copper intakes are normal and would have no deleterious effects except for the fact that excess copper retention in the sheep is favoured by extremely low molybdenum levels in the herbage; and in the third area copper intakes are normal and molybdenum is low although not extremely so, but the ingestion of the plant *Heliotropium europeum*, containing hepatoxic alkaloids, induces liver damage. The liver damage results in an excess concentration of stored copper in a reduced number of liver cells from which it is readily released to produce the haemolytic crisis characteristic of the disease.

One further example will be given. In parts of England an ataxic condition affecting newborn lambs occurs. This condition is known as 'swayback' and appears clinically and pathologically identical with the enzootic neonatal ataxia which occurs in lambs in parts of Australia and South Africa. This latter disease has been shown to be an expression of simple copper deficiency in the animal arising from subnormal levels of copper in the soils and herbage of the affected areas. In England, however, the same copper deficiency state occurs on soils and pastures normal in copper content. Since the animals are undoubtedly lacking in copper, it is obvious that the affected herbage must either contain some factor which adversely affects copper utilisation by the animal or be lacking in some factor which normally promotes its utilisation. Despite extensive research which has eliminated several possibilities, including molybdenum, the 'conditioning' factor or factors have yet to be disclosed.

CAUSES OF DEFICIENCIES AND EXCESSES

Deficiency diseases and intoxications of grazing stock develop almost exclusively as a consequence of either inadequate or excessive intakes of particular nutrients from the food itself or of an imbalance among these food nutrients. Contrary to popular belief, water supplies rarely contribute significant quantities of minerals or any other nutrients affecting the health and productivity of stock under naturally-occurring conditions. An exception is provided in endemic fluorosis areas, where the drinking water is generally the major source of the excess fluoride. In regions where highly saline waters are consumed, animal health and production may also be adversely affected. Even with iodine, despite the consistent and significant differences in iodine content between the potable waters of goitrous and nongoitrous regions and the value of the iodine levels in the drinking water as an index of the iodine status of an area, over 90% of the total daily intake of this element comes from the food. This has been shown to apply in goitrous and nongoitrous areas alike. The causes of deficiencies and excesses in grazing stock must therefore be sought in the factors determining the nutrient content of herbage plants.

The nutritive value of all plants depends basically upon the species, or strain and upon the soil and climatic conditions under which they have grown. These factors are interrelated and are capable of modification by man and by the grazing animal itself. It is through modifications of these basic factors, by grazing management, by irrigation, by the use of soil amendments and by fertiliser treatment, that control of the nutritional disabilities of the grazings of particularareas is most widely achieved.

The chemical composition of all herbage plants reflects to varying degrees the nature of the soil upon which they grow. Plants react to a lack of an available mineral in a soil either by limiting their growth, by reducing the concentration of the mineral in their tissues or by both. Similarly, plants respond to high available levels in the soil either by increasing growth, by raising the levels of the minerals in their tissues or by both. The extent to which one or other of these processes actually occurs, varies enormously with different plant species, with different minerals and with the seasonal or climatic conditions of growth. The status of the soil with respect to available levels of various minerals is probably the most important single factor determining the concentrations of those minerals in individual herbage species. Nevertheless, it is essential to appreciate that the soil-plant-animal mineral sequence is a matter of extreme complexity and that the levels of minerals in plants and those of other nutrients essential to animals can be profoundly influenced by factors other than type of soil.

Botanical effects are perhaps the most spectacular. Thus certain species of *Astragalus* and other genera have long been known to have the ability to accumulate excessive concentrations of selenium. On soils high in selenium, levels of 3000–5000 ppm occur in these species compared with 10–30 ppm in more common herbage species (15). Moreover, these accumulator species have the capacity to absorb forms of selenium in the soil which cannot be absorbed by othr types of plants and to convert them into forms which can be so absorbed (3). In this way, areas safe or marginal in selenium status for the animal can be converted into seleniferous areas. On present evidence, accumulator or convertor plants of this nature play little part in other naturally-occurring conditions but comparatively little is yet known of inherent species or strain differences in the chemical composition of herbage plants and in their varying capacity to respond to a range of soil and climatic conditions.

Evidence obtained in recent years suggests that such species or strain differences may be of more significance than has generally been assumed. Thus certain plant species have recently been identified in England which carry incredibly high concentrations of strontium, up to 26,000 ppm, compared with 10–100 ppm in most other species growing in the same soils (4). New Zealand

research has revealed 20-fold differences in iodine uptake by pasture species and 10-fold differences among strains of rye-grass grown together on the same soil types (10). Plant iodine concentrations, on the basis of the New Zealand findings, depend more upon the species or strain than upon soil type, soil iodine content, or season.

The characteristic differences in composition between legumes and grasses, especially the substantially higher levels of nitrogen (protein), calcium and carotene (provitamin A) of the former, have the widest significance of all. The seasonal protein deficiency in grazing animals which occurs so consistently in tropical and sub-tropical regions throughout the world can largely be ascribed to the lack of suitable legumes. In many areas this deficiency is accentuated by climatic disadvantages which permit only a short growing period in the herbage grasses and impose a long dormant period in each year when the grazing is mature, fibrous and of low or very low protein content. Conditions such as these prevail over huge areas, including much of northern Australia. Levels of 3%, or less, crude protein in the herbage dry matter, causing severe protein deficiency, have been shown to be the principal factor limiting animal production for several months in each year in these areas (5). Moreover, this deficiency occurs primarily as a consequence of the climatic disabilities of the region and only secondarily as a reflection of the low soil nitrogen status.

Phosphorus deficiency is undoubtedly the most widespread qualitative nutritional disability affecting grazing cattle, other than protein deficiency. The 2 deficiencies are, in fact, intimately linked, largely because both the protein and the phosphorus levels in herbage plants fall markedly with maturity and remain low as long as the herbage is dry and mature. Thus the crude protein and the phosphorus contents of Mitchell grass (*Astrebla spp.*) pastures growing in norther Australia have been shown to fall from approximately 16% and 0.2%, respectively, in the young, green stage, to 3.2% and 0.05%, respectively, when dry and mature (6). Whilst low soil phosphorus levels have been well recognised for many years as a cause of low phosphorus concentrations in plant tissues, the dominating influence of season and climate upon these concentrations has been less appreciated. In any area the relative lengths of the dry, dormant periods (low herbage phosphorus) and of the growing periods (high or relatively high herbage phosphorus) can determine the occurrence or otherwise of phosphorus deficiency in the grazing animal. The much higher incidence of phosphorus deficiency in grazing cattle than in sheep can be explained partly in terms of the lower requirements of the latter per unit of dry matter consumed and partly in terms of their more selective grazing habits. With both animal species, however, climatic conditions, as well as soil conditions, can markedly influence phosphorus intakes from either natural or improved grazings.

The incidence of phosphorus (and calcium) deficiency in grazing stock may also be influenced by another climatic component—sunlight. The animal's ability to assimilate these elements and to cope with adverse Ca:P ratios in the fodder are known to be greatly improved under conditions of liberal vitamin D supply. Since field herbage and its conserved products, hay and silage, are not normally rich sources of this vitamin, the extent of vitamin D formation in the animal's skin becomes an important determinant of calcium and phosphorus utilisation. Over most of Australasia and South Africa, grazing animals are exposed, even in the winter, to sufficient irradiation to ensure adequate vitamin D synthesis and prevent signs of avitaminosis D. Where the animals are housed during the winter, as in northern Europe and America, and when they graze in areas where the sun altitude is about 35° or less, so that very few of the short ultraviolet rays vital for vitamin D synthesis in the animal's body survive atmospheric absorption and reach the earth's surface, this source of vitamin D is largely denied the animal and a deficiency of vitamin D can be expected. Even in Australia and New Zealand, rickets in young sheep responding to vitamin D therapy has been reported in certain seasons (8, 9). It seems likely, also, that some of the early differences between European and South African workers in respect to minimum calcium and phosphorus requirements and to the significance of high Ca:P ratios can be explained in terms of variable vitamin D synthesis in the animals.

DETECTION OF NUTRITIONAL DISABILITIES

The appearance of specific lesions or abnormalities in the animal has long been used to define the limiting factors in endemic diseases of nutritional origin. The occurrence of fragile and deformed bones and spontaneous fractures provided the first clues to calcium and phosphorus deficiency in cattle in the early European studies and a depraved appetite or pica, expressed as a craving for bones (osteophagia) gave a lead in the classical South African studies of phosphorus deficiency in veldt cattle. The development of enlarged thyroids or goitres in animals has been used as a pointer to iodine deficiency for very many years and, in more recent times, the appearance of straight, stringy wool minus its characteristic crimp has become a valuable and sensitive index of copper deficiency in Merino sheep. Detailed clinical and pathological observations, in fact, are essential diagnostic tools in the study of all nutritional deficiencies and excesses.

It is nevertheless important to appreciate their limitations. In the first place not all nutritional disorders have characteristic and specific symptoms, as was pointed out with respect to cobalt deficiency. Secondly, the deficiency or toxicity may be so mild in its incidence that such vague and nonspecific signs as unthriftiness, depressed appetite, or subnormal growth and productivity may be the only discernible disabilities. Thirdly, the clinically-obvious functional or structural

disorder may be merely the final expression of a defect arising earlier in a chain of metabolic events and may bear no simple or obvious relationship to the limiting element or nutrient. For instance, the anaemia which occurs in 'bush-sickness' was logically interpreted by the early New Zealand workers as evidence of iron deficiency—an interpretation supported by the responses obtained in bush-sick animals by dosing with crude iron salts and ores. It was many years before Australian scientists were able to show that the anaemia was actually an expression of cobalt deficiency and that the crude iron compounds were effective because of the minute amounts of cobalt that they carried.

For these reasons chemical analysis of soils, of pastures and fodders, and of animal tissues and fluids have become indispensable aids in detecting and defining nutritional disorders in livestock and in delineating the environmental limits under which they are likely to occur. In general terms, soil analysis is the least satisfactory means of defining deficient or toxic areas and appropriate analyses of body tissues or fluids the most satisfactory, although either can be misleading. A range of physical and chemical methods capable of estimating, with great delicacy and precision, the mineral elements in soils and in plant and animal tissues has been developed over the last few years and there is evidence that we are on the eve of still more promising analytical techniques, especially for the trace elements. The importance of such techniques to the diagnosis and understanding of nutritional disorders in animals can hardly be overemphasised. The spectrograph has played a key role in providing early leads to the solution of a number of such problems, not merely because of its convenience, specificity and accuracy, when properly used, but because it allows a range of elements to be estimated at the same time and therefore direct comparisons to be made of their relative paucity or richness in healthy and 'affected' soils, plants or animals. It was, in fact, spectrographic examinations of this type which gave the first vital clues to the causes of enzootic ataxia of lambs (copper deficiency) and of 'teart' of cattle (molybdenum toxicity) and to the role of molybdenum in limiting copper retention in sheep.

Despite considerable efforts in many countries to correlate soil type and soil mineral status with the incidence of mineral abnormalities in grazing stock, the results have mostly been disappointing. This is hardly surprising because, in addition to the many factors which influence the utilisation of herbage minerals by animals, the uptake of minerals from the soil by plants is itself a matter of great complexity, in which the level of the mineral in the soil, as determined by chemical analysis, or even the level of so-called 'available' mineral in the soil, is only one of the factors involved. Nevertheless, in particular regions and with particular soil types, the correlation can be high, as the Scottish workers have shown with their use of 2.5% acetic acid extractable cobalt as an index of cobalt deficiency areas (13). Also, where correlations of this kind have been established, soil surveys can be of great value in mapping the extent and location of problem areas and in drawing attention to their likely occurrence elsewhere.

Herbage analyses, as might be expected, are generally more satisfactory than soil analyses, as they bring the investigator 1 step nearer the animal, and some success has been achieved in setting up standards, or more usually ranges of pasture concentrations of several minerals which can be classified as healthy, marginal, or 'affected' from the animal viewpoint. Diagnoses based on herbage analysis alone are not always reliable, however, because *a*) the samples analysed may not represent the material actually eaten by the animal—an important factor with such selective feeders as sheep, *b*) different pasture species and strains can vary markedly in nutrient content, even when growing together, as has already been pointed out in respect to selenium, iodine, copper and cobalt, and *c*) the level of a given mineral in the herbage is only one of the determinants of deficiency or toxicity states in the animal. For instance, both the minimum and the maximum levels of pasture copper compatible with full health and productivity in grazing stock vary with the concentrations of molybdenum and inorganic sulphate present and with those of other constituents not yet fully defined. In fact, in one part of Australia, pastures containing 6 ppm copper on the dry basis have been found fully satisfactory for growth, well-being and wool production in sheep, whereas in another part a minimum of 10 ppm is necessary for the same purpose.

Chemical analysis or biological assay of the tissues and fluids of the animal, especially of the classical substrates blood and liver, have become invaluable aids in the detection of nutritional abnormalities in grazing stock. The blood or serum tends to reflect in its composition the nutritional status of the animal with respect to a number of nutrients including vitamin A, phosphorus and copper. Determination of the level of these substances in the blood has become a routine diagnostic procedure and useful standards of adequacy have been established. It should be remembered, however, that when endogenous supplies of the element are available to the animal from body stores or tissue katabolism, blood levels can at least temporarily be an unreliable guide. This applies in regard to both vitamin A and copper because the liver, in each case, has considerable capacity for storage. It applies also to phosphorus where lack of available dietary energy or protein results in substantial loss of body weight. Under such conditions the blood can draw sufficient phosphorus from tissue breakdown to mask an inadequacy in the diet and to maintain inorganic serum phosphate levels for many weeks.

Blood cobalt determinations, because of their difficulty, have been little used as diagnostic aids. The concentration of cobalt in the liver, on the other hand, has been greatly used because it is much easier to estimate and is markedly influenced by the level of dietary cobalt intake. Extensive New Zealand studies have

established that 0.04–0.06 ppm Co, or less, in the dry matter of the livers of sheep and cattle indicate cobalt deficiency and that 0.08–0.12 ppm, or more, indicate a satisfactory cobalt status (12). Since cobalt deficiency in ruminants is essentially an induced vitamin B_{12} deficiency, it seemed that vitamin B_{12} assays of liver would provide a more logical and direct approach to correct diagnosis. Tentative liver vitamin B_{12} criteria based on results from numerous field trials have now been worked out and are in routine use in New Zealand (2).

The levels of certain elements in the urine, notably of iodine, fluorine and selenium, have also been employed as aids in assessing the nutritional status of animals with respect to these elements, although they are fraught with considerable hazards because of the body's variable capacity to retain them in certain of its tissues. With selenium a much simpler and more satisfactory diagnostic test is the determination of the selenium content of the hair. Although individual variability is large and seasonal changes are marked, South Dakota workers have found that selenium estimations of hair clippings from grazing cattle provide a reasonable prediction of the safety of a given area from the point of view of possible selenosis (14).

It is apparent from the foregoing that neither clinical observations of the animal nor estimations of nutrient levels in soils, in pastures or in animal tissues by themselves constitute satisfactory criteria of the status of an area in regard to actual or likely incidence of nutritional abnormalities in grazing stock. When these are used together, however, and their combined evidence assessed by the discerning investigator, rapid and revealing data for recognising and predicting both deficiency and toxicity states can be obtained, even where they are mild. Nevertheless, it is the animal which is always the final arbiter of its environment. The ultimate criterion of a deficiency or a toxicity is the improvement in health, growth or production which occurs in response to changes in the intake or the utilisation by the animal of the elements or nutrients concerned.

PREVENTION AND CONTROL MEASURES

Highly successful procedures for the prevention and control of most of the naturally-occurring deficiencies and toxicities in grazing stock have now been developed. The procedure of choice varies greatly with the element or nutrient in question and is influenced by both physiological and economic considerations and by the general husbandry conditions which prevail.

With mineral deficiencies occurring under extensive conditions, direct treatment of the animal is normally practised, rather than indirect supplementation through appropriate fertiliser treatment of the soil or pasture, because the latter procedure can be uneconomic as a consequence of high transport and application costs and of low herbage productivity per unit area brought about by other limiting factors such as inadequate rainfall or low winter temperatures. Furthermore, the effectiveness of fertiliser treatment in raising the mineral content of the herbage to adequate levels can be adversely affected, as in phosphorus deficiency, by the dominant influence of dry periods on the phosphorus levels in plants and, as in iodine deficiency, by the unreliable and fluctuating uptake of iodine by plants.

For the above reasons direct treatment of the animal raised under sparse grazing deficiency conditions is likely to be the most satisfactory. This is normally achieved either by i) the provision of mineral licks containing the deficient element, which is common with phosphorus, iodine, copper and cobalt, ii) by oral dosing or drenching, which is common with copper, cobalt, and vitamins A and D, or iii) by injections at appropriate intervals. This is becoming increasingly practised in copper deficient areas since slowly absorbed organic complexes such as copper glycinate have become available. Mineral licks represent the easiest means of treatment but have the disadvantage that there is no certainty that all animals will consume enough at all times; whilst oral drenches, although they give certain control, are costly in time and labour especially where frequent dosing is essential. With nutrients such as copper and vitamin A, which are readily stored in the body to provide reserves against periods of inadequate intake, drenching with large doses several months apart is satisfactory. In fact, under Australian conditions, a single massive dose of vitamin A, as high as 500,000 IU, given to sheep early in the dry period as a 'booster' to existing liver reserves of this vitamin, has been found to provide complete protection against avitaminosis A. The success of intramuscular injections of organic complexes of copper in providing protection against copper deficiency in grazing cattle for as long as 6 months is also due to the body's capacity to utilise stored copper, as well as to the slow absorption which takes place from these compounds.

The nutritional physiology of cobalt is so different from that of other elements that prevention and control procedures are more exacting. The animal body has an exceedingly limited capacity to store cobalt and, in any case, tissue cobalt cannot pass readily to the rumen and reticulum where its physiological action resides. The ruminant requires regular supplies of this element in its rumen in order that the bacteria present can synthesise the host's requirements for vitamin B_{12}. Injections of cobalt are therefore valueless and oral dosing must be regular and frequent. Weekly drenches supplying 7 mg cobalt per dose per sheep have been found completely satisfactory, whereas dosing with larger amounts at longer intervals gives partial but not complete protection. The disadvantages of such frequent handling of grazing animals led the South Australian workers to develop the 'cobalt bullet' (7). This is a small, heavy pellet of cobalt oxide and clay which, when administered to sheep, lodges in the rumeno-reticular region and, because of its density, remains there to yield a small steady supply of cobalt to the rumen organisms respons-

ible for vitamin B_{12} synthesis. This ingenious device is still in the process of further development and improvement but already gives promise of providing a cheap and effective form of control of cobalt deficiency in both sheep and cattle. Whether a similar technique can be used with other elements, such as copper, is as yet unknown.

Although stress has rightly been placed on deficiency prevention and control measures involving direct treatment of animals raised under sparse or relatively sparse grazing conditions, it should not be assumed that these are necessarily the most desirable procedures where animal husbandry practices are more intense and economic considerations therefore less overriding. Where such conditions exist, these control procedures may be adopted, and indeed frequently are, with spectacular success, but other opportunities for improving the quality and the quantity of the grazing arise. Under these more favourable conditions, appropriate fertiliser treatment of the soil to raise the levels of particular nutrients in the herbage is more generally practised, particularly when, as with phosphorus and copper applications on some soil types, this also increases the growth of the pastures, so improving the general nutritional status of the area. In some cases, also, the remedying of a soil deficiency which affects plant growth only, results in an improvement in quality, as well as the quantity, of the herbage. Thus in molybdenum-deficient areas, molybdenum applications as low as 1 oz. per acre can increase pasture production several hundred per cent. Such applications usually also increase significantly the over-all protein content of the mixed herbage. Furthermore, direct application of the deficient mineral to the soil or plant may not always be necessary. The uptake of certain minerals by plants can be profoundly modified by the use of soil amendments such as lime or sulphur, which respectively raise or lower the soil pH. In molybdenum toxicity areas this can be of great importance since plants absorb this element much less readily under acid than under alkaline soil conditions.

The naturally-occurring toxicities in grazing stock are rather more difficult to prevent or to cure than are the deficiencies, especially when they occur under extensive conditions and are accentuated, as in a high proportion of the seleniferous areas of the world, by the presence of accumulator and convertor plants. Arsenic supplementation of the animal's diet or drinking water has been found highly protective against selenosis in animals under pen-feeding conditions, but has obvious hazards under range conditions. In endemic fluorosis areas also, the only practical form of protection is periodic removal of the animals from dependence upon the fluorided waters. In areas where molybdenosis or chronic copper poisoning develop, by contrast, a high degree of control can be achieved by modification of the dietary copper-molybdenum ratio. Thus massive dosage of cattle with copper has long been known to prevent the disease teart, which is due to very high intakes of molybdenum from the herbage. Moderate dosage with copper or treatment of the pastures with copper to raise their content of this element to normal levels is completely protective against 'peat scours,' which is a molybdenosis conditioned by copper deficiency; and the provision of molybdate licks to sheep liable to chronic copper poisoning reduces or eliminates the incidence of this malady by reducing copper retention in the animal.

GENERAL CONCLUSION

Nutritional disabilities affecting the productivity of grazing stock are known to occur over large areas of the earth's surface. As diagnostic techniques improve and other factors limiting animal production are overcome, it can be predicted that many more such areas will be discovered, especially those in which the qualitative abnormalities in the herbage are so slight that acute disease conditions with well-marked symptoms rarely arise.

It is becoming increasingly apparent that naturally-occurring deficiencies or excesses in animals seldom exist in simple or uncomplicated form, or as a direct and simple consequence of a deficiency or excess of a particular element in the soil. They invariably occur as a result of a complex interaction between the soil, the plant, the climatic environment and the animal itself. Sometimes 1 and sometimes another of these 4 factors is the dominant causal component of the complex.

For these reasons, both the detection and definition of nutritional disabilities in grazing stock and the procedures to be used in their prevention or control require that the whole environment of the animal be taken into account. The application of existing techniques for the detection and the control of deficiency and toxicity states in grazing animals, in which the significance of the whole soil-plant-climate-animal interrelationship is recognised, offers enormous scope for increasing food production, through better animal health and productivity. Despite the spectacular advances of recent years, it seems likely that we are only at the beginning of their real potential for increasing world food production.

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Qualität und Quantität der vom Wiederkäuer stammenden Nahrungsmittel, ausser Milch

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DIE NACHFOLGENDEN AUSFÜHRUNGEN sollen sich in das allgemein zur Diskussion stehende Thema: "Tierernährung und Nahrungsmittelproduktion" einordnen. Deshalb werden die vielseitigen Beziehungen zwischen der Fütterung des Wiederkäuers und der Fleischerzeugung durch den Wiederkäuer im Vordergrund stehen. Eine gewisse Einschränkung von Seiten des Referenten wird insofern nötig, als bei allen Betrachtungen wiederum der große Wiederkäuer, das Rind, vorrangig behandelt wird.

Schaf und Ziege tragen unter extensiven landwirtschaftlichen Produktionsbedingungen, auch unterschiedlich vom Standort mehr oder weniger—mehr in Australien, Neuseeland, weniger im kontinentalen Europa—zur Fleischversorgung der Bevölkerung bei. Der große Wiederkäuer besitzt jedoch im persönlichen Wirkungsbereich des Referenten die bei weitem größere wirtschaftliche Bedeutung. Zudem dürften für Schaf und Ziege ähnliche, wenn nicht die gleichen Gesetzmäßigkeiten zwischen Fütterungsgeschehen und dem schließlichen Nahrungsmittel, dem Fleisch und Fett, bestehen, wie das im Folgenden für das Rind ausgeführt wird. Analogieschlüsse in dieser Richtung erscheinen im Hinblick auf den gleichen anatomischen Aufbau und die funktionell gleichsinnig ablaufenden Verdauungsvorgänge bei allen landwirtschaftlich genutzten Wiederkäuerarten gerechtfertigt.

Eine Reihe von Gesichtspunkten möchten hier als besonders wichtig für die Erzeugung vom Wiederkäuer stammender Nahrungsmittel herausgestellt sein.

1) 'ABSOLUTE' FUTTERSTOFFE.

Ein Großteil der im landwirtschaftlichen Betrieb erzeugten Futterstoffe ist nur durch den Wiederkäuer entweder für Milchproduktion oder für die Fleischbildung rentabel zu verwerten. Man kann in diesem Zusammenhang von *absolutem Wiederkäuer-futter* sprechen. Hierzu gehören

a) alle rohfaserreichen und

b) alle voluminösen Futterstoffe,

letztere in etwas abgeschwächtem Sinn, d. h. nahezu sämtliche Rauh-, Grün- und Saftfuttermittel des landwirtschaftlichen Betriebes.

Lediglich ein rohfaserarmes Grünfutter oder daraus hergestellte Silage findet in kleineren Mengen Eingang in die Schweinefütterung bei vergleichbar guter Nettoenergieausbeute.

Im allgemeinen aber können nur die Vormägen des Wiederkäuers mit ihrer vielgestaltigen Mikrobenflora rohfaserreiche und deshalb schwer verdauliche Futterstoffe mit hinreichend hohem Nutzeffekt verwerten, d. h. einen wesentlichen Anteil der im Futterstoff vorhandenen Bruttoenergie (Kalorimeterwärme) in Nettoenergie überführen. Nur die Nettoenergie kann für den Erhaltungsstoffwechsel oder zur Bildung körpereigener Substanz (Protein, Fett) herangezogen werden.

Der Einhufer (Pferd), das über einen zwar nicht anatomisch aber funktionell ähnlich eingerichteten Verdauungstrakt wie der Wiederkäuer verfügt, kann in der Verwertung rohfaserreicher und voluminöser Nahrungsstoffe mit dem Rind konkurrieren, fällt aber als Nahrungsmittellieferant weitgehend aus. Er besitzt auch sonst nur noch geringe wirtschaftliche Bedeutung im landwirtschaftlichen Betrieb.

2) HANDELSFUTTERMITTEL

Neben den sog; absoluten Futterstoffen für den Wiederkäuer können auch Handelsfuttermittel (Getreide, Ölkuchenschrote, Trockenschnitzel u. a.) für die Rindermast von Bedeutung sein. Sie werden mit hohem Nutzen neben absoluten Futterstoffen eingesetzt und vom Wiederkäuer häufig ähnlich gut oder besser verwertet als in der Geflügelfütterung. Wesentliche Ausnahmen hierzu bilden das Mais- und Weizenkorn, die beide durch das Huhn wegen eines eigentümlichen und nur für diese Tierart spezifischen hohen Nettoenergiegehaltes besser verwertet werden als durch den Wiederkäuer. Diese Ausnahmen bestehen nicht mehr für Mühlennachprodukte (z. B. Weizenkleie).

Bei der Ausnutzung von Ölkuchenschroten (Soja, Erdnuß, Baumwollsaat) für die Fleischproduktion bestehen ebenfalls keine wesentlichen Unterschiede zwischen beiden Tierarten.

3) NETTOENERGIEVERWERTUNG

Der Anteil der aus einem Futterstoff verfügbaren Nettoenergie (Stärkewert, Cal., Therms), der auf den Erhaltungsstoffwechsel des Wiederkäuers entfällt und

damit dem eigentlichen Prokuktionsprozess für Fleisch und Fett bei der Rindermast verlorengeht, beträgt durchschnittlich 58–60%. Diese Zahlen gelten über den gesamten, heute wirtschaftlich interessanten Mastbereich zwischen 100–500 kg Lebendgewicht des Fleischrindes.

Der auf den Erhaltungsstoffwechsel entfallende Verlust an Nettoenergie ist somit keineswegs größer, als bei einem Masthuhn (1) und wesentlich kleiner als bei der Legehenne. Die Legehenne wendet 75–80% der zugeführten 'produktiven Energie' (=netto) für den Erhaltungsstoffwechsel auf. Dementsprechend werden nur 20–25% der Nettoenergie in der Eimasse für den menschlichen Verbrauch zurückgewonnen. Bei der Rindermast sind es annähernd 40% der Nettoenergie, die sich im Nahrungsmittel wiederfinden.

Umgekehrt ist die Wiederkäuermast, vom Standpunkt der Energiebilanz aus betrachtet, der Produktion von Milcherzeugnissen unterlegen. Bei 10 Liter täglicher Milchleistung wandern etwa 50% der aufgewendeten Nettoenergie in die Milch, bei täglich 15 Liter Milchleistung sind es bereits 60–70% der zugeführten Nettoenergie, die sich im Milchprodukt wiederfinden, gleich schwere Tiere vorausgesetzt.

Ein entsprechender, zahlenmäßiger Vergleich mit Erzeugung von Schweinefleisch ist schwer durchzuführen, weil hier die Beurteilung des Futters nach seinem Nettoenergiewert allgemein nicht üblich ist. Ausreichende Zahlenunterlagen fehlen. Trotzdem läßt sich hier

a) aus dem, verglichen mit dem Wiederkäuer um 50% höheren Fettproduktionsvermögen der isolierten Nährstoffe (Eiweiß, Fett, Stärke, Zucker, mit Ausnahme der Rohfaser) (2)

b) aus den hohen täglichen Gewichtszunahmen, die im Energiewert nicht hinter den Zunahmen bei der Rindermast zurückstehen, im Gegensatz dazu aber bei wesentlich kleinerem Körpergewicht erreicht werden, schließen, daß das Schwein in der anteilsmäßigen Ausnutzung der zur Verfügung stehenden Nettoenergie für Produktionszwecke dem Mastrind überlegen ist.

4) PANSENFUNKTION

Der Wiederkäuer ist wegen der synthetischen Leistungen seiner Mikrobenflora im Vormagensystem weitgehend unabhängig von der Eiweißqualität in der für ihn bestimmten Futtermischung. Der Begriff der "Eiweißwertigkeit" in der Fütterung spielt für den Wiederkäuer nur eine sehr untergeordnete Rolle.

Bis zu einem Drittel kann sein täglicher Eiweißbedarf mit synthetisch zugänglichem Amidstickstoff (Harnstoff) gedeckt werden. Der Wiederkäuer nimmt mit dieser Verwertungsmöglichkeit eine Sonderstellung unter allen landwirtschaftlichen Nutztieren ein. Ebenso erwähnenswert ist die Unabhängigkeit des Wiederkäuers von der Zufuhr wasserlöslicher Vitamine (B-Komplex) mit der Nahrung. Auch hier greift er zur Bedarfdeckung auf die synthetischen Leistungen anwesender Vormagen- und Darmsymbionten zurück.

5) FLEISCHQUALITÄT

Der Maßstab zur Beurteilung der Fleischqualität richtet sich auch bei der Rindermast nach den Verbraucherwünschen. "Was dem Konsumenten am besten gefällt, ist die beste Qualität". Verbraucherwünsche vor allem im Hinblick auf das Fleisch: Fett-Verhältnis sind wechselnd und langfristigen Schwankungen unterworfen. Sie hängen u. a. von der jeweiligen Versorgungslage mit anderen Grundnahrungsmitteln wie Kohlehydraten und Fetten, ab (Notzeiten! Zeiten des Überflusses!). Es ist deshalb schwer, den Begriff einer "erwünschten" Fleischqualität über längere Zeiträume hinweg zu definieren. Gesicherte Korrelationen zwischen Verbraucherwünschen und physikalisch-chemischen Untersuchungsdaten zu finden, ist nur in Einzelfällen gelungen.

Von ausschlaggebender Bedeutung bei der Qualitätsbeurteilung von Rindfleisch sind:

a) Geruch Geschmack, Farbe
b) das Fleisch: Fett-Verhältnis
c) das Safthaltevermögen des Muskelfleisches
d) das Wasserbindungsvermögen des Muskelbreis
e) Wassergehalt und Konsistenz.

Für die Bestimmung des Handelswertes (3) spielen die aufgeführten Qualitätsfaktoren eine wesentliche Rolle.

Ein übergang von Geruchs- und Geschmacksstoffen aus Futtermitteln in das spätere, tischfertige Lebensmittel (Fleisch und Fett) kann allgemein negiert werden. Manche peroral oder parenteral verabreichten Arzneimittel können, soweit sie fettlöslich sind, zu einer geruchlichen und geschmacklichen Beeinträchtigung des Lebensmittels führen (z. B. Narkotika). Sie stehen aber außerhalb des normalen Fütterungsgeschehens.

Das Fleisch: Fett-Verhältnis beim heranwachsenden Wiederkäuer wird weniger durch die Fütterung beeinflußt, als vielmehr durch das "Eiweißansatzvermögen" des Tieres bestimmt. Dieses ist vornehmlich von genetischen Faktoren abhängig und fällt außerdem mit zunehmendem Alter ab. Beim sehr jungen Wiederkäuer allerdings (Kälbermast bis 120 kg) ist ein deutlicher Einfluß der Fütterung auf das Fleisch: Fett-Verhältnis vorhanden.

Ein gutes Safthaltevermögen der Muskulatur ist für die küchenmäßige Verarbeitung zusammenhängender Muskelpartien ein ausschlaggebender Faktor (Koch- und Bratproben).

Ein größeres Wasserbindungsvermögen des zerkleinerten Muskelbreis ist bei der Verarbeitung des Rindfleisches zu Brüh- und Kochwurst von Bedeutung. Hierfür eignet sich insbesondere das Fleisch des Jungbullen. Zur Rohwursttherstellung (Dauerwurst, Salami) wird das trockene Fleisch älterer Kühe bevorzugt. Es ist hier dem saftigen Fleisch des Jungbullen überlegen.

Ochsenfleisch ist wegen der intramuskulären Fetteinlagerung (Marmorierung) das vielerorts bevorzugte Küchenfleisch (Suppenfleisch, Steakfleisch).

6) BEVORZUGTE MASTRICHTUNGEN IN DEUTSCHLAND

Die im Rahmen einer Kälbermast bzw. Jungbullenmast vom Tier produzierten Nahrungsmittel entsprechen dem zur Zeit in Deutschland geltenden Qualitätsbegriff für Kalb- oder Rindfleisch besser als Produkte aus der Ochsenmast. Die bei der Ochsenmast über 300 kg nachlassenden täglichen Gewichtszunahmen beruhen zum wesentlichen Teil auf der vermehrten, vom Verbraucher aber nicht gewünschten Fettbildung. Es ist wenig wahrscheinlich, daß sich das kastrierte männliche Jungtier in der anteilsmäßigen (Erhaltungsstoffwechsel: Produktionsstoffwechsel, vgl. Ziffer 3) Ausnutzung der angebotenen Nettoenergie vom Jungbullen unterscheidet. Geringere Gewichtszunahmen des Ochsen im Mastabschnitt von 350–500 kg beruhen vielmehr auf dem geringeren Wassergehalt des angesetzten Körperfettes im Vergleich zum Muskelfleisch und ebenso in der stärkeren Zusammendrängung des Energiegehaltes in der Gewichtseinheit Fett verglichen mit dem Muskelprotein.

7) FUTTERZUSATZSTOFFE (FEED ADDITIVES)

Von besonderer Bedeutung ist heute der mutmaßliche Einfluß von Wirkstoffzusätzen in der Fütterung (Feed additives) auf die Fleischqualität, insbesondere im Hinblick auf die "hygienische Unbedenklichkeit" der unter solchem Einfluß entstandenen Nahrungsmittel Es kann keinem Zweifel unterliegen, daß alle heute üblichen Eingriffe in dieser Richtung vom toxikologischen Standpunkt als unbedenklich angesehen werden können. Gemeint ist der Einsatz von Vitaminen, Antibiotika, Tranquilizern, aber auch Hormonen der Sexualsphäre.

Die Barriere, die dem Fremdstoff (Feed additive) über das landwirtschaftliche Nutztier als Stoffwechsel- und Entgiftungsfilter immer gesetzt ist, stellt in der Regel einen wirksamen und sicheren Schutz für den Verbraucher so entstandener Nahrungsmittel dar. Eine momentane Beunruhigung des Lebensmittelkonsumenten, die von Kontinentaleuropa, vielleicht sogar der Deutschen Bundesrepublik ausgehend, weite Teile der Welt einschließlich die U. S. erfaßt hat, wird wieder abklingen. Das Risiko, das den Menschen als Lebensmittelverbraucher bisher mit dem Einsatz von Futtermittelzusätzen in toxikologischer Hinsicht verbindet, ist so gering, daß es sich mathematisch kaum fassen läßt und ist dementsprechend in statistischer Sicht nicht vorhanden. Bedenklich allein sind Beunruhigung und psychische Noxen, die der Lebensmittelverbraucher durch die fortgesetzte Diskussion toxikologisch nicht existierender Probleme erfährt; nicht dagegen die Verwendung der bisher bekannten Wirkstoffe in der Tierernährung als solche. Stichhaltige Gründe, die den Einsatz auch nur eines einzigen, heute üblichen Futtermittelzusatzes bei der Ernährung unserer landwirtschaftlichen Nutztiere verbieten würden, liegen nach Ansicht des Referenten nicht vor.

Umgekehrt muß auf den Verbleib und die mögliche Kumulation von Insektiziden und Herbiziden im Gewebe des landwirtschaftlichen Nutztieres (hier: Wiederkäuer) geachtet werden. Bei diesen Stoffen handelt es sich jedoch nicht um Feed additives, sondern um eine sekundäre Verschmutzung von Futterstoffen (Feed contaminants). Aber auch hier muß zunächst ein entscheidendes Stoffwechsel- und Entgiftungsfilter, dargestellt durch das landwirtschaftliche Nutztier, passiert werden, bevor eine Gefährdung des Lebensmittelverbrauchers eintreten kann.

Ad 1, 2 et 6:

Wenn als bestes Maß für die Produktionsleistung eines Futtermittels sein Gehalt an Nettoenergie gelten darf, hat der Referent die Freude, als Kernstück seines Beitrages zum obigen Thema folgende Formel als optimale Fütterungsnorm für Kälber und Jungrinder im Mastabschnitt zwischen 50–500 kg vorzutragen:

1% + 500 bis 800 Stärkeeinheiten

Diese Formel wurde 1957 von L. Krüger (4) in Deutschland aus langjährigen Erfahrungen heraus publiziert. Sie hat sich seitdem in zahlreichen Mast- und Aufzuchtversuchen bewährt und stellt heute die Faustregel für die Rindermast in Deutschland dar. Sie besagt, daß bei der Kälberaufzucht und -mast, aber auch bei der darüber hinaus fortgeführten Mast von Jungrindern die folgende Regel gilt:

Die optimale Zufuhr an Nettoenergie

1000 Stärkeeinheiten (St.E.) = 2360 Cal Nettoenergie
= 2,36 Therms, Net Energy

richtet sich nach dem jeweiligen Körpergewicht des Wiederkäuers. Täglich sind 1 % des Körpergewichts (kg) × 1000 St.E. zu füttern und zusätzlich dazu 500–800 St.E.

Die Zahl zon 500 St.E. gilt dabei für die Aufzucht, der Wert von 800 zusätzlichen St. E. für die intensive Mast (Kälbermast, Jungrindermast).

In den einzelnen Mastabschnitten kann dabei je nach Futterart diese Norm um ± 500 St.E. unter- oder überschritten werden.

Ins praktische Beispiel übertragen bedeutet die Formel, daß ein zur intensiven Mast aufgestellter Jungbulle von 200 kg Lebengewicht ein tägliches Futterangebot von

2000 + 800 = 2 800 St. E.

erhalten soll, um eine maximale Gewichtszunahme zu zeigen. Eine Erhöhung dieser Fütterungsnorm über 2 800 St. E. hinaus bleibt ohne Einfluß auf die Gewichtsentwicklung des Tieres (5).

Die folgenden Verhältnisse sind bei der Zusammenstellung von Futterrationen zu beachten:

Verd. Eiweiß: St. E.	Lebendgewicht	
1:4	(bis 100 kg)	Kälbermast
1:5	(100 bis 150 kg)	Jungrindermast
1:6	(250 bis 400 kg)	Jungrindermast
1:7	(400 bis 500 kg)	Jungrindermast

Im Gewichtsabschnitt bis 100 kg (Kälbermast) nimmt das Kalb bei eingeschränkter Vollmilchgabe (bis 150 kg) nur dann genügend St. E. auf, wenn 1 kg Trockenfutter (z. B. Milchaustauschfutter oder sonstiges Kraftfutter) mehr als 700 St. E. pro kg enthält. In den späteren Mastabschnitten gilt die Beziehung

St. E.: Trockenmasse = mindestens 1:2,0 bis 2,3
St. E.: Rohfaser = 1: 0,5

Fütterungsplan für den Rindermastabschnitt 100–500 kg:

Grundfutter:	Gärfutter, Grünfutter (Weide) Heu und Rüben.
Kraftfuttergabe täglich:	1 kg Getreidemischung (bis 2,5 kg ansteigend) 1 kg Trockenschnitzel 1 kg Heu (bis 5 kg ansteigend)
Mineralstoffe: täglich	50–100 g Mineralstoffmischung (12% P_2O_5) (bei Stallfütterung angereichert mit 40 000 I. E. Vitamin D/kg).
Ø Tägliche Zunahmen:	900–1100 g
Nährstoffverbrauch/kg Zunahme: optim.	3200–3500 St.E.

Diese Zahlen, die aus empirischen Fütterungsversuchen gewonnen wurden, bestätigen hier für den Wiederkäuer, daß die optimale Energieaufnahme eines Masttieres über seine Futtermischung in einem nahezu konstanten Verhältnis zu seinem Erhaltungsumsatz steht. Dieser richtet sich nach der jeweiligen "Stoffwechselgröße" (= metabolic size = 72 × kg Lebendgewicht 0,75). (6)

Die Tabelle gibt an, welche Anteile der nach der Krüger'schen Fütterungsnorm (vereinfacht in 1 % + 600) aufgewendeten Nettoenergie für den Erhaltungsstoffwechsel (maintenance requirement) in der Rindermast verbraucht werden:

Die Tabelle zeigt, daß unabhängig vom jeweiligen Lebendgewicht des Jungrindes ein annähernd konstantes Verhältnis besteht zwischen der für den Erhaltungsstoffwechsel aufgewendeten Nettoenergie und der für die Produktion von Nahrungsmitteln verbleibenden Nettoenergieanteile.

Ungefähr 40% der im Futter enthaltenen Nettoenergie gehen bei der Rindermast in das später vom Menschen genutzte Nahrungsmittel über. Mit großer Wahrscheinlichkeit gilt dies auch für den Jungochsen (vgl. unter 6), obgleich hier die Lebendgewichtszunahmen infolge vermehrter Fettbildung geringer sind.

Damit hat die aus praktischen Erfahrungen entwickelte Fütterungsnorm von Krüger, die inzwischen

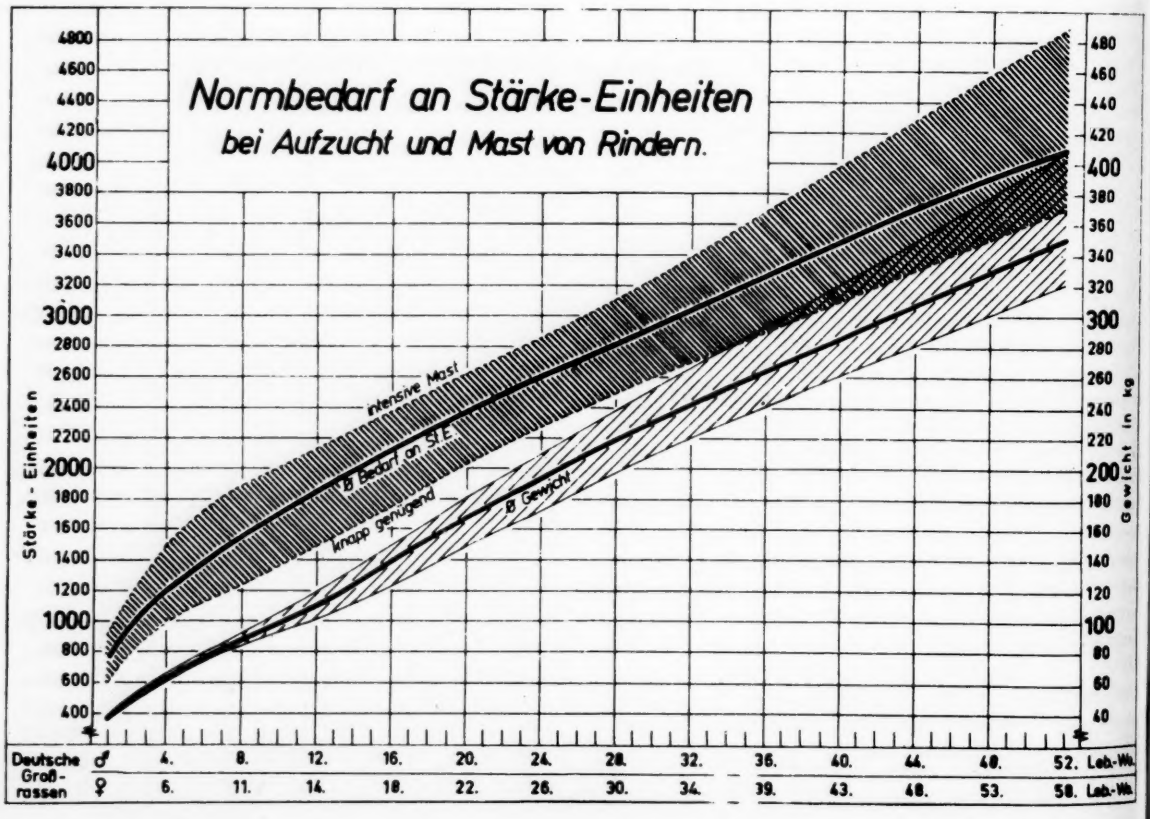

TABELLE I

Lebendgewicht kg (Wiederkäuer)	Fütterungsnorm (in St. E.) 1% ± 600 St. E.	Fütterungsnorm in Cal Nettoenergie	Erhaltungsbedarf in Cal (72 × Gew. in kg$^{0.75}$)	Erhaltungsbedarf in Cal × 100 / zugeführte Nettoenergie in Cal
100	1 600	3 770	2 275	60,2
200	2 600	6 130	3 830	62,4
300	3 600	8 490	5 190	61,1
400	4 600	10 850	6 430	59,3
500	5 600	13 210	7 590	57,5

von Witt und Richter in Deutschland bestätigt wurde, für den Wiederkäuer die diesbezüglichen mehr theoretischen Überlegungen von Kleiber (7) im Jahre 1933 bestätigt. Sie rechtfertigt erneut das Denken des Ernährungsphysiologen in Nettoproduktionswerten, in Nettoenergiewerten von Futtermischungen.

Auf die Futterwertbeurteilung nach THERMS N.E. übertragen, verändert sich die Formel wie folgt:

1% Körpergewicht (in kg) × 2,360 Therms + 1,2 bis 1,9 Therms
oder:
1% Körpergewicht (in lb) × 1,07 Therms + 1,2 bis 1,9 Therms

Nur mit Einschränkungen jedoch ist sie auf die hierzulande (USA) übliche TDN - Bewertung von Futterstoffen zu übertragen, weil eine mathematisch feste Beziehung zwischen verd. Gesamtnährstoff (TDN) eines Futterstoffes und seinem Nettoproduktionswert (St. E.) ganz sicher nicht besteht. Nur der von Oscar Kellner geprägte Begriff der "Wertigkeit" eines Futterstoffes vermag den Unterschied zwischen TDN-Wert und dem tatsächlich im Respirationsversuch gefundenen Nettoenergiewert eines Futterstoffes zu überbrücken.

Ad 4:

Ergänzend hierzu wird ausgeführt, daß es nur dem Wiederkäuer gelingt, über die synthetischen Leistungen seiner Vormagenflora Eiweißstoffe pflanzlicher Herkunft mit oft niedriger "biologischer Wertigkeit" in qualitativ hochwertiges Muskelprotein zu überführen. Auch im quantitativen Sinne besitzt der Wiederkäuer nicht das scharf definierte Eiweißbedürfnis anderer landwirtschaftlich genutzter Tierarten. In gewisser Hinsicht macht er keinen Protein-, sondern lediglich einen Stickstoff- (N) -bedarf geltend. Auf die Zufuhr ausreichender Energiemengen (Kohlehydrate, Zellulose) ist bei der Harnstoffbeifütterung zu achten, desgleichen auf ein genügendes Sulfat- (S) -angebot. Die Eiweißsynthese durch Bakterien verbraucht Energie, die sich zum Großteil im gebildeten Protein wiederfindet. Das S-Angebot ist bei der mikrobiellen Synthese der schwefelhaltigen Aminosäuren (Methionin, Cystein) häufig der begrenzende Faktor.

Bei der Erörterung der Produktionsmöglichkeiten vom Tier stammender Nahrungsmittel verdient diese Ausnahmestellung des Wiederkäuers besondere Beachtung.

Ad 5 et 6

Eine besondere Eigenart des mitteleuropäischen Wirtschaftsraumes liegt hier in der Bevorzugung des hellfarbigen Kalbfleisches durch den Konsumenten. Es ist dies das "weiße Fleisch" vom Mastkalb in der Gewichtsklasse bis 110 kg.

Die beliebte weiße Farbe des Kalbfleisches wird umso sicherer erreicht, je später die Pansentätigkeit beim Tier einsetzt, d. h. je länger die Verdauungsvorgänge des Kalbes dem eines monogastrischen Tieres ähneln. Wird das Kalb frühzeitig zum funktionell vollentwickelten Wiederkäuer, was bei Gewöhnung an rohfaserreiche Futterstoffe schon im Alter von 4–5 Wochen gelingt, verliert das Fleisch seinen hellroten, fast weißen Farbton und ist nur schwer absatzfähig.

Die Fütterung des Mastkalbes berücksichtigt diese Tatsachen. Mastkalbernährung ist Säuglingsernährung. Große Vollmilchgaben garantieren i.a. beste Fleischqualität. Eine gute Kalbfleischqualität geht mit der Entwicklung von Nierenfett einher. Bei der Qualitätsbeurteilung des Schlachtkalbes ist daher die Einbettung beider Nieren in üppiges Fettgewebe von großer Bedeutung. Ausschließliche Vollmilchmast (600–800 kg pro Tier) ist wirtschaftlich aber unrentabel. Das "Milchaustauschfutter" mit hohen Trockenmagermilchanteilen (60%) ist an ihre Stelle getreten. Eine gute bis sehr gute Fleischqualität kann auf diesem Wege erzielt werden. Kälbermast kann auch unter Beibehaltung kleiner Vollmilchmengen (60–150 kg) mit flüssiger, häufig dick-sauer gelegter Magermilch erfolgreich betrieben werden. Es empfiehlt sich dann, den Energiegehalt der Magermilch durch Zusatz von leichtverdaulichen Kohlehydraten oder Fetten (Schlachtfette, pflanzliche Öle) zu erhöhen.

Je stärker die Vollmilchgaben bei der Kälbermast zurücktreten, umso mehr gewinnt die Aufwertung der täglichen Futterration mit Wirkstoffen (Vitamine A, D, Antibiotika) an Bedeutung. 10 000 I. E. Vitamin A, 800–1 000 I. E. Vitamin D, 15–20 mg Vitamin E pro Tier und Tag sichern den Masterfolg. Kleine tägliche Antibiotikagaben (40–60 mg) helfen mit, unerwünschte Kälberdurchfälle zu verhindern, und verbessern im allgemeinen die täglichen Gewichtszunahmen.

Bei ausschließlicher Magermilchmast werden Spitzenleistungen in der Fleischqualität selten erzielt. Vor allem die Ausbildung des Nierenfettes beim Schlachttier ist spärlich. Trotzdem steigt mit weitgehender Einsparung von Vollmilch die Rentabilität der Mast erheblich. Die erzielte Fleischqualität ist durchaus befriedigend. Die Schlachtqualität steht ebenfalls in enger Beziehung mit dem störungsfreien Mastablauf. Komplikationen durch länger anhaltende Indigestionen oder sonstige Erkrankungen haben einen deutlich negativen Einfluß auf die Fleischqualität.

Bei allen Mastverfahren sollen die täglichen Gewichtszunahmen 800–1 000 g betragen. Das Endgewicht des Mastkalbes von 100 kg wird durchschnittlich nach 56 bis 60 Tagen erreicht.

Ad 5 et 7:

Mit Ausnahme der vorerwähnten Verhältnisse beim Mastkalb scheint ein wesentlicher Einfluß von Seiten der Fütterung auf die Fleischqualität des älteren Tieres nicht zu bestehen. Voraussetzung ist allerdings, daß dem Muskelproduktionsvermögen des heranwachsenden Wiederkäuers im Stickstoffangebot mit der Nahrung ausreichend Rechnung getragen wird. Bei einem zu weiten Eiweiß: St. E.-Verhältnis kann es zu vorzeitigem und unerwünschten Fettansatz kommen, was den heutigen Qualitätsansprüchen (Fleisch: Fettverhältnis) zuwiderläuft.

Im allgemeinen setzt zunächst die Fettbildung in den Körperhöhlen ein (Darmfett, Nierenfett). Im Anschluß daran entstehen die Fettpolster der Körperoberfläche (subcutanes Fett). Erst dann erfolgt die Fetteinlagerung zwischen den einzelnen Muskelbündeln. Dieses intermuskuläre Fett gibt dem Muskel ein "marmoriertes" Aussehen. Solches Fleisch wird bei der küchenmäßigen Zubereitung bevorzugt. Trotzdem kann der "Handelswert" des Schlachtkörpers wegen eines verengerten Fleisch: Fettverhältnisses durch starke Ausbildung wenig gefragter Fettdepots in den Körperhöhlen und im Unterhautbindegewebe absinken.

Der Einfluß von Futterstoffen auf die spezifische Zusammensetzung und Konsistenz des Körperfettes ist bei der Rindermast weit weniger gegeben als bei der Milchfettbildung. Überwiegende Grünfütterung (Weidegang) führt zu stärkerer Ablagerung von gelben Pflanzenpigmenten im Rinderfett als zu Zeiten der pigmentarmen Winterfütterung.

Der Wassergehalt der Muskulatur ist ebenfalls weitgehend fütterungsunabhängig. Mit dem Alter der Schlachttiere fällt der Wassergehalt im Muskelfleisch ab. Dies trifft für das Rind ebenso wie für das Schwein zu. Als korrespondierende Größe nimmt der inter-bzw. intramuskuläre Fettgehalt zu. Die häufig vertretene Auffassung, daß wasserreiche Futterstoffe (z. B. Kartoffel- oder Getreideschlempe, Biertreber) zu einer vermehrten Wassereinlagerung in das Muskelgewebe führen, hat der wiederholten experimentellen Nachprüfung nicht standgehalten (3). Der Wassergehalt der Muskulatur bestimmt im wesentlichen die Konsistenz des Fleisches.

Safthaltevermögen und Wasserbindungsfähigkeit der Muskulatur sind wenig über die Fütterung zu beeinflussen. Sie hängen vorrangig von Lebensalter, Geschlecht und genetischen Faktoren ab. Über "Preßproben" zur Ermittlung des Safthaltevermögens sowie über die Extraktion des fibrillären Muskeleiweißes (Myosin) als brauchbares Maß für das Wasserbindungsvermögen ist kürzlich berichtet worden (8).

Das Wasserbindungsvermögen des Muskels ändert sich mit pH-Wert und Lage des isoelektrischen Punktes im Muskelprotein. Bei Weidegang soll der isoelektrische Punkt verschoben sein, was eine Verschlechterung der Wasserbindungsfähigkeit nach sich zieht. (9)

Die Zähigkeit (das Gegenteil von Zartheit = tenderness) des Muskelfleisches nimmt mit der Einlagerung von Bindegewebe in die Muskulatur zu. Die Zähigkeit soll mit dem Oxyprolingehalt des Bindegewebes korrelieren. Das reine Muskeleiweiß enthält diese Aminosäure offenbar nicht. Um den Einfluß von Kastration, Alter und Fütterung auf die Fleischqualität zu prüfen, ist wiederholt der Gehalt von Oxyprolin im Fleisch untersucht worden (10).

Obgleich der Vitamingehalt der vom Tier stammenden Fleisch- und Fetteile letztlich vom Vitaminangebot in der Nahrung abhängt, gelingt eine gezielte Anreicherung über die Fütterung nicht. Eine Ausnahme bildet der Vitamin A-, Carotin- und Vitamin E-Gehalt der Wiederkäuerleber. Er steigt bei reichlichem Carotin- und Tocopherolangebot während der Sommerfütterung in der Leber an. Besonders der Carotingehalt der Wiederkäuerleber sinkt umgekehrt im Winter ab (11). Die starke Vitamin A-Aufwertung von Milchaustauschfutter macht sich im Vitamin A-Gehalt der Kalbsleber ebenfalls bemerkbar, was als deutliche Qualitätsverbesserung zu werten ist.

Der Einfluß anderer Wirkstoffzusätze in der Futterration richtet sich dagegen vornehmlich auf die Quantität der erzeugten Nahrungsmittel. Die Qualität wird wenig, aber i. a. auch nicht negativ beeinflußt.

Sexualhormone verschiedenen Typs werden in der Rindermast (vorwiegend 300–500 kg) mit gutem Erfolg eingesetzt. Im wesentlichen handelt es sich um oestrogen wirksame Stilbenabkömmlinge, wie Diäthylstilboestrol (DS), Hexoestrol (H) oder um Testosteron (T) als männliches Keimdrüsenhormon.

Bei täglicher oraler Zufuhr von DS (10 mg) oder T (20–40 mg) oder auch durch einmalige Inplantation von H oder DA (ca. 30 mg) lassen sich signifikante Mehrzunahmen bei Färsen und Jungochsen bei gleichzeitig verbesserter Futterverwertung erzielen. (12) (13).

Schlachtausbeute und Fleischqualität bleiben dabei unbeeinflußt. Nur vereinzelt ist bei Verwendung von DS über eine geringfügig vermehrte Wassereinlagerung in die Muskulatur berichtet worden, so kürzlich bei Mastversuchen mit Schafen (14). Auf einen geringfügigen Abfall der Fleischqualität nach Stilboestrolinplantation bei Färsen (nicht bei Ochsen) hat Klostermann (15) hingewiesen. Es sind dies jedoch Ausnahmebefunde.

Kastrate, die gegenüber Jungbullen im Mastabschnitt von 300–500 kg (16) um etwa 15% kleinere Tageszunahmen und eine schlechtere Futterverwertung (15–20%) zeigen, sprechen auf die Zufuhr von Sexualhormonen im allgemeinen günstiger an als Jungbullen. Aber auch in der Jungbullenmast kann mit einer deutlichen Mehrzunahme gerechnet werden, wenn die tägliche Zufuhr von DS 20 mg beträgt (17).

Bei fachgerechter Dosierung in der Jungrindermast sind Muskulatur und Fett des Schlachtkörpers frei von Hormonrückständen. Sehr kleine und toxikologisch unbedenkliche Mengen sind in Leber und Niere mit stark verfeinerten biologischen Untersuchungsmethoden gelegentlich aufgefunden worden (5–15 mcg/kg!) (17).

Die günstige Wirkung von Tranquilizern auf Gewichtszunahme und Futterverwertung bei der Rindermast (z. B. 2,5 mg Hydroxycin oder 60 mcg Reserpin täglich) wurde in den letzten Jahren wiederholt beschrieben (17, 18). Auch hier war ein Einfluß auf die Fleischqualität nicht festzustellen. Rückstände im Schlachtkörper konnten nicht festgestellt werden (17).

Antioxydantien (Butylhydroxytoluol, Tocopherole, Gallate) werden bei der Stabilisierung von Schlachtfetten im Milchaustauschfutter bei der Kälbermast mancherorts eingesetzt. Sie scheinen jedoch keinen Einfluß auf die Qualität des fertigen Lebensmittels zu nehmen. Ihr möglicher Übergang in das gebildete Körperfett ist nur in Ausnahmefällen beim Schwein diskutiert worden. Die Haltbarkeit von Schweineschmalz konnte so auf indirektem Wege verlängert werden (19).

Die Bedeutung der Fütterungsantibiotika (Aureomycin, Terramycin) in der Wiederkäuerernährung beschränkt sich im wesentlichen auf die Kälberaufzucht und mast, wo die Breitspektren-Antibiotika wichtige Funktionen übernehmen (vgl. unter 6). Eine günstige Auswirkung der Antibiotikabeifütterung in späteren Mastabschnitten des Wiederkäuers wurde aber ebenfalls beobachtet (18).

Tetraalkylammoniumstearat (TAS) scheint sich als "Sicherungszusatz" in der Wiederkäuerfütterung zu bewähren. Es verhindert das Auftreten von Tympanien (bloat) bei der Rindermast, die besonders bei überwiegender Grünfütterung mit Leguminosen oder auch sonst bei eiweißreicher Kozentratfütterung ohne ausreichende Rauhfuttergaben gefürchtet sind. Mehrere Angaben finden sich über verbesserte Gewichtszunahmen bei der Schaf- und Rindermast nach täglicher Verabreichung von TAS (200–400 mg TAS/Rind/Tag) (20, 21).

Demgegenüber ist allgemein die Verwendung von Schilddrüsenhemmstoffen, (Thiouracil, Methylthiouracil), in der Rindermast umstritten. Trotzdem liegen bei der Ausmast (finishing) von Kühen und Ochsen (500–600 kg) eine Reihe günstiger Resultate bezüglich Gewichtszunahmen und Futterverwertung vor. (22). Eine Beeinflusung der Fleischqualität durch antithyreoidale Substanzen im ungünstigen Sinne wurde nicht beobachtet. Insbesondere ließ sich eine stärkere Wasserretention der Versuchstiere nicht aufzeigen. Thiouracil-Rückstände im Gewebe konnten nicht gefunden werden (23). Holländische Autoren führen die Mehrzunahmen allerdings zum Teil auf eine Vergrößerung und stärkere Füllung des Pansens zurück (24).

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Quantity and quality of final products other than milk—nonruminants (pigs)

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DURING THE PAST 20 to 25 years there has been, especially in the western world, an increased tendency for consumers to demand more lean meat and less lard. This is no doubt due to the improvement in the standard of living, mechanization, which reduces manual labor, and to a lesser extent, the current discussion about the influence of animal fats on the general health of man. The task at hand must be, therefore, to an ever increasing extent, to produce pigs which have as much meat and as little fat as possible.

A very great number of experiments concerning the influence of nutrition on carcass quality, as reviewed by Fevrier (21), have been carried out in many countries under very different conditions. Due to the limitation of the report, it is impossible to refer to all the results obtained. Unfortunately, it has also been necessary to exclude important questions, such as the influence of minerals, vitamins, antibiotics and hormones on meat quantity. A major problem connected with the appraisal of many of the results is the variations in the methods of evaluating carcass quality, applied in different countries and by different scientists. Not only do methods of measuring and of judging carcass quality differ, as reviewed by Harrington (23), but so do those of feeding intensity, protein levels, carcass weights and carcass qualities reported by Clausen and Gerwig (10). As sex and carcass weight are of great influence on carcass quality, experiments without sex and weight corrections may contribute to the difficulties in evaluating the results.

The following attempt at showing the main principles in the production of lean pigs through some examples is therefore, to a fairly great extent, based upon Danish experiments, because the carcass evaluation has been the same in each experiment and therefore, the results are directly comparable.

MEAT QUANTITY

General Principles for the Production of Lean Pigs

I. No pig can produce meat to the maximum level determined by its genes without being fed a sufficient quantity of protein. This is shown in table 1.

By increasing the level of protein to 1.5 kilos skim milk and 110 grams protein concentrate per pig per day, weight gain increases, feed consumption per kilo gain decreases, and the carcass quality is considerably improved (19).

II. No pig can be forced, through exceptionally high levels of protein in the feed, to produce more meat than that limited by its genes. This is shown in tables 2 and 3.

High levels of protein supplementation, above 1.5 kilo skim milk and 110 grams protein concentrate per pig per day, has neither at limited, nor at semi ad lib. feeding, increased the daily gain, decreased the feed consumption per kilo gain or improved the carcass quality (11).

From principles I and II follows that whether or not an influence on carcass quality is to be obtained from feeding increasing quantities of protein will depend partly on the level of protein fed to the pigs and partly on the pigs' limitative genes for meat production (meat or lard type).

Selection of boars and sows with the best possible genetic ability for producing meat carcasses is, therefore, of the utmost importance. When progress is made, the protein requirements of the breed increases.

Protein supplements are expensive. It is important to feed enough to ensure maximum meat production, but excess protein means losses to the producer. In Denmark, pig rations contain approximately 14–15% crude protein or 11–12% digestible true protein for a live pig weighing 20 to 30 kilos, decreasing to 12–13 and 9–10%, respectively, at a live weight of 90 kilos. This is obtained by giving a constant daily protein supplement (skim milk, skim milk plus protein concentrates or only protein concentrates) combined with increasing quantities of basic feed (cereals, cereals plus roots or potatoes).

III. When the daily requirement for maintenance and meat production have been covered, the rest of the daily feed intake will be utilized for fat production, that is, the higher the daily intake of energy, the fatter the pig.

In 1940, McMeekan (37) showed the influence of the level of nutrition on the form and composition of the bacon pig. At the levels of nutrition High-High, High to a live weight of 100 lb. and then Low, Low to a live weight of 50 lb. and then High, and Low during the

TABLE 1. *Influence of Feeding Increasing Quantities of Protein on the Meatiness of Pigs**

Skim milk/pig/day, kilos	0.5	1.0	1.5
Protein concentrate/pig/day, gm†	40	75	110
Basic feed: barley	up to level for limited feeding		
Daily gain, g	446	497	513
S.F.U./kilo gain‡	4.08	3.68	3.57
Av. thickness of back fat, cm§	3.78	3.31	3.42
Slight of lean-measurement, cm	3.86	3.44	3.37
Area of meat in cross section, cm^2	29.3	33.1	34.2
Area of fat in cross section, cm^2	51.4	45.5	44.2
Fat in % of meat in cross section	175	137	129

* Growing period 20 to 90 kilos. Limited feeding. Aver. of 4 experiments. 16 pigs in each treatment group. † ⅔ extr. soybean-oil meal and ⅓ meat and bone meal. ‡ S.F.U. = Scandinavian feed units. 1 S.F.U. = feeding value of 1 kilo barley (at 14% moisture). § Estimation of carcass quality, see Clausen (9).

TABLE 2. *Influence on Thickness of Back Fat of Feeding High Levels of Protein**

Skim milk/pig/day, kilo	1.5	3.0	1.5	2.25	3.0
Protein concentrate/pig/day, g†	110	225	110	168	225
Basic feed: barley	up to level for limited feeding		semi ad lib.		
Daily weight gain, g	646	624	807	824	800
S.F.U./kilo live weight gain	3.16	3.39	3.32	3.28	3.38
Av. thickness of back fat, cm	3.28	3.27	3.53	3.56	3.54
Weight of leaf fat, kilo	1.81	1.86	1.69	1.78	1.70

* Growing period 20 to 90 kilos. First 2 columns av. of 3 experiments, the last 3 columns av. of 3 experiments. 12 and 22 pigs, respectively, in each treatment group. † ⅔ extr. soybean-oil meal and ⅓ meat and bone meal.

TABLE 3. *Influence on Meat Content of Carcass of Feeding Increasing Quantities of Protein**

Protein level	Low	Average	High
Skim milk/pig/day, kilos	0.75	1.50	2.1
Protein concentrate/pig/day, gm†	55	110	155
Av. daily feed consumption/pig in S.F.U.	1.89	1.91	1.88
Per cent meat in carcass‡	53.7	57.3	56.9

* Growing period 20 to 90 kilos. 8 individually fed pigs in each group. † ⅔ extr. soybean-oil meal and ⅓ meat and bone meal. ‡ Without head, leaf fat and entrails.

TABLE 4. *Limited Feeding Versus ad Lib. Feeding from Self-Feeders**

	Limited Feeding	Ad Lib. Feeding from Self-Feeders
Av. back fat thickness in cm	3.14	3.60
Per cent pigs in grade I	90	43

* Automatic self-feeders closed 2 days weekly. 96 pigs in each treatment group.

TABLE 5. *Restricted Feeding Versus Semi ad Libitum Feeding by Hand**

	Restricted Feeding	Semi ad Lib. by Hand
S.F.U./pig/day†	1.84	2.17
Digestible true protein/pig/day, g	188	209
Daily weight gain, g	573	654
Growing period 20 to 90 kilos, days	122	107
S.F.U./kilo live weight gain	3.22	3.33
Av. thickness of back fat, cm	3.28	3.57
Thickness of back fat (mid-back), cm	2.35	2.59
Slight of lean-measurement, cm	2.87	3.17
Fat in % of meat in cross section	112	128
Per cent pigs in grade I	79	52

* Growing period 20 to 90 kilos. 618 and 609 pigs, respectively, in the 2 treatment groups. † See footnotes table 1.

TABLE 6. *Effect of Increasing Daily Feed Intake**

S.F.U./pig/day	1.68	1.89	2.18	2.48	2.60
Daily weight gain, g	536	621	699	762	790
Growing period 20 to 90 kilos, days	131	113	100	92	89
S.F.U./kilo gain	3.14	3.06	3.13	3.27	3.31
Thickness of back fat, cm	3.18	3.24	3.39	3.69	3.71
% lean meat in carcass	58.1	56.0	54.1	51.8	51.8

* 24 individually fed pigs in each group.

whole growing period, he found a back fat thickness of 3.29, 2.70, 3.99 and 2.24 cm, respectively.

Before, but especially after the publication of these results, many scientists had already studied this problem. During the past 16 years, large scale experiments on this subject have been carried out in Denmark (13, 15–19). Some of the results are summarized in tables 4 to 6.

The conclusion must be: Until the genotype for meat production of pigs in general has been improved enough to allow ad lib. feeding the feed intake of the pigs must be limited, i.e. when production aims at lean animals.

Methods for Limited Feeding

The following definitions for the level of nutrition are now generally accepted: 'Feeding ad libitum' (ad lib.) = feeding according to appetite from self-feeders; 'feeding semi ad libitum' (semi ad lib.) = feeding according to appetite, by hand, 2 to 3 times daily, but the pigs must empty the trough within approximately 20 to 30 minutes; 'limited (restricted) feeding' = feeding, by hand, according to live weight or age, the feed being weighed out daily according to a fixed scale.

Limited feeding requires more labour and reduces the yearly turnover in the piggery, but it decreases feed consumption per unit of gain and improves the carcass quality. This system is, therefore, recommended in several countries, although the feeding scales may differ from one country to another according to breed and aim of production. In Denmark approximately 230

TABLE 7. *Influence of Different Protein Supplements on Carcass Quality*

Protein Supplement/Pig/Day:	Fat in % of Meat in Cross Section
3 kilos of skim milk	106
1 kilo skim milk plus 150 g protein concentrate I	111
1 kilo skim milk plus 100 g protein concentrate II plus either 45 g extr. peanut-oil meal or 60 g extr. sunflower-seed meal	112
1 kilo skim milk plus 50 g meat and bone meal plus either 90 g extr. peanut-oil meal or 120 g extr. sunflower-seed meal	112
225 g protein concentrate I	118
150 g protein concentrate II plus either 70 g extr. peanut-oil meal or 90 g extr. sunflower-seed meal	123
75 g meat and bone meal plus 180 g extr. sunflower-seed meal	130
75 g meat and bone meal plus 140 g extr. peanut-oil meal	144

Protein concentrate I: ⅔ extr. soybean-oil meal and ⅓ meat and bone meal. Protein concentrate II: ½ extr. soybean-oil meal and ½ meat and bone meal.

pig-feeding societies have been established. Assistants paid by the producers visit the farms each fortnight, weigh 2 to 3 average pigs in each pen and advise the farmer on rations to be given according to the fixed scale. Breirem *et al.* (1) report similar satisfactory results from using a fixed scale according to the pigs' age.

Many authors have emphasized that limited feeding can be attained even if ad lib. feeding is practiced, when the ration is 'diluted' by a 'filler' having either a high content of crude fibre or a low content of dry matter.

The results can be summarized as follows: At ad lib. feeding the 'fillers' have reduced the daily energy intake and therefore also the speed of growth. The thickness of back fat is reduced, but at the same time the back fat becomes softer. When 'fibre-fillers' must be used in quantities sufficiently large to reduce back fat thickness down to the level achieved at limited feeding, feed consumption per unit of gain increases to such an extent that payment for the 'filler' is negligible. Great interest must, therefore, be directed towards self-feeders, which can weigh out daily rations with sufficient accuracy to meet the fixed scale recommended by limited feeding.

Influence on Carcass Quality of the Amino Acid Content in Total Feed

Kropf *et al.* (29) compared two 16% protein rations, 1 with a good amino acid balance, the other with a poor one. They obtained a better carcass quality when using the first type. A 12% ration with a good amino acid balance produced better carcasses than the 16% ration with poor amino acid balance.

The amino acid balance of the total ration is influenced partly by the protein supplements, partly by the basic feed (cereal mixtures, roughage, etc.) of the pigs. This is shown in the following results from Danish experiments (20). Table 7 gives a summary of the influence of 'different protein supplements' on carcass quality being expressed as fat in per cent of meat in the cross section at the last rib. All the pigs were fed limited according to weight, and the daily consumption of Scandinavian feed units (S.F.U.) and digestible true protein were approximately the same for all groups.

Table 8 shows the influence on carcass quality of changing a cereal mixture (barley replaced by increasing amounts of milocorn (sorghum) or maize (corn)) when the daily protein supplementation consisting of skim milk and/or protein concentrates is kept constant.

The results shown in tables 7 and 8 demonstrate the importance of using feed which is well balanced with respect to the amino acid content. Protein nutrition is essentially amino acid nutrition, and several experiments have been carried out, as reviewed by Madsen (36), to assess the pigs' requirements of essential amino acids. However, the effects of adding different amino acids or different quantities of amino acids to a fixed ration have nearly all been tested on growth rate and, to some extent, on feed consumed per unit of gain. Unfortunately, the daily weight gain and the feed conversion rate, as shown in table 9, tell very little about the carcass quality.

All 3 groups consumed the same quantity of feed per day (limited feeding). The pigs in group 2 (2 kilos of skim milk replaced by 150 g of protein concentrate) grew faster and had a better feed conversion rate than those in group 1 which were fed 3 kilos of milk per day,

TABLE 8. *Influence on Carcass Quality of Replacing Barley by Maize or Milocorn*

	Maize			Milocorn	
Protein supplement/pig/day:					
Skim milk, kilos	3.0	1.0	0	1.5	0
Meat and bone meal, g	0	50	75	37	75
Extr. soybean-oil meal, g	0	100	150	73	150
	Fat in % of meat in cross section:				
Cereal mixture:					
100% barley	105	107	112	108	121
80% barley, 20% maize or milo	108	118	119	110	123
60% barley, 40% maize or milo	111	120	126	119	138
40% barley, 60% maize or milo	116	125	136	132	150

TABLE 9. *Influence of Three Different Protein Supplements on Gain, Feed Conversion and Carcass Quality**

Skim milk/pig/day, kilos	3.0	1.0	0
Protein concentrate, day, gm†	0	150	225
No. of pigs	87	50	47
S.F.U./pig/day	1.83	1.84	1.83
Daily weight gain, g	532	545	535
S.F.U./kilo live weight gain	3.44	3.38	3.42
Av. thickness of back fat, cm	3.10	3.18	3.29
Slight of lean-measurement, cm	2.78	3.07	3.15
Area of meat in cross section, cm^2	36.4	35.9	34.6
Area of fat in cross section, cm^2	37.4	39.5	41.7
Fat in % of meat in cross section	103	110	120

* Growing period 20 to 90 kilos. † ⅔ extr. soybean-oil meal and ⅓ meat and bone meal.

TABLE 10. *Methionine Supplementation of a Milk-Cereal Diet (Preliminary Results)*

Level of nutrition	Limited				Semi Ad Lib.			
% DL-methionine added to cereal mixture	0	0.1	0.2	0.3	0	0.1	0.2	0.3
Growing period 20 to 90 kilos live weight								
S.F.U./pig/day	1.83	1.87	1.83	1.86	2.42	2.39	2.45	2.39
Av. daily gain, g	597	611	596	584	700	786	753	717
S.F.U./kilo gain	3.10	3.08	3.09	3.19	3.46	3.05	3.30	3.36
Thickness of back fat, cm	2.97	2.91	3.08	3.25	3.68	3.56	3.47	3.46
Slight of lean-measurements, cm	2.95	2.90	2.90	3.15	4.40	2.95	3.30	3.40
Score (0–15) for lean meat in carcass	11.3	12.3	12.0	11.8	6.5	12.3	9.5	9.3

but the carcasses were fatter. The pigs in group 3 (3 kilos of skim milk replaced by 225 g of protein concentrate) had approximately the same daily gain and feed conversion rate as the pigs in group 1, but the carcasses were much fatter. Daily gain and feed conversion rate do not always show the biological value of feeds with respect to carcass quality.

The amino acid requirements of pigs must therefore be defined as 'the quantity of and the balance between the different amino acids which ensure maximum meat production in the carcass.'

Effect on Carcass Quality by Adding Lysine and/or Methionine to Different Rations

As shown in table 7, the best carcass quality of the Danish Landrace pig was obtained when 3 kilos of skim milk were fed per pig per day and cereals up to the level of limited feeding. It is, however, not known whether this ration contains sufficient lysine and methionine. In a pilot experiment Clausen *et al.* (20) supplemented the milk-cereal ration (85% barley and 15% oats) with 0.1, 0.2 and 0.3% DL-methionine mixed with the cereal. Half of the pigs were fed limited, the other half semi ad lib. The results (table 10) indicate that the addition of 0.1% methionine improved carcass quality, whereas 0.2 and 0.3% methionine had an adverse influence on daily gain, feed conversion rates and carcass quality.

When 3 kilos of skim milk per day are replaced by 225 g of a protein concentrate (⅔ extr. soybean-oil meal and ⅓ meat and bone meal) and barley is given up to the level of limited feeding, the content of methionine and lysine in the total ratio decreases. In an experiment, the results of which are given in table 11, the 2 amino acids were added to the protein concentrate-barley ration so that the total content of lysine, methionine and lysine plus methionine in the ration were identical to the milk-barley ration.

TABLE 11. *Methionine and Lysine Supplementation of a Protein Concentrate-Barley Diet (Preliminary Results)*

Skim milk, kilos/pig/day	3.0	0	0	0	0
Protein concentrate, g/pig/day*	0	225	225	225	225
L-lysine added to ration			+		+
DL-methionine added to ration				+	+
Av. thickness of back fat, cm	3.04	3.33	2.98	3.26	3.31
Slight of lean-measurement, cm	2.60	2.82	2.48	2.68	2.63
% meat in carcass	59.5	57.1	59.0	59.9	58.9

* ⅔ extr. soybean-oil meal and ⅓ meat and bone meal.

TABLE 12. *Methionine and Lysine Supplementation of a Barley-Milocorn Diet (Preliminary Results)*

Protein conc., g/pig/day*	225	225	225	225	225
% barley in cereal mixture	100	40	40	40	40
% milocorn in cereal mixture	0	60	60	60	60
Lysine added to ration			+		+
Methionine added to ration				+	+
Av. thickness of back fat, cm	3.01	3.79	3.61	3.44	3.20
Slight of lean-measurement, cm	2.25	3.75	3.65	3.55	3.70
% meat in carcass	57.6	54.2	54.9	55.1	56.0

* ⅔ extr. soybean-oil meal and ⅓ meat and bone meal.

The supplementation of the protein concentrate-barley ration by lysine improved the carcass quality, whereas methionine or methionine plus lysine failed to bring about any further improvement. Nielsen (39) found that a supplementation of corn-soy and barley-soy diets by 1 lb. lysine HCL/ton decreased back fat thickness and increased the percentage of lean cuts.

When barley is replaced by milocorn at unchanged protein supplementation (table 8), the content of methionine and lysine decreases in the total ration. Table 12 shows the results of an experiment, in which the 2 amino acids were added to a ration consisting of 225 g protein concentrate per pig per day and a cereal mixture of 40% barley and 60% milocorn. Such amounts of methionine and/or lysine were added to the barley-milo ration so that the content of the 2 amino acids in the total ration was equivalent to that of the ration of group 1 viz. barley plus protein concentrate.

The supplementation of the barley-milo ration by methionine and lysine improved the carcass quality but this did not fully reach the level found when feeding 100% barley.

In a similar experiment (table 13) so much methionine and lysine were added to a grain mixture consist-

ing of 40% barley and 60% maize that the total content of the 2 amino acids in the ration was equivalent to that of the group which was fed barley as the only cereal.

The supplementation of the barley-maize diet by methionine and lysine improved the carcass quality. The response was slight when the protein supplementation was given in skim milk, greater when a mixture of extr. soybean-oil meal and meat and bone meal was used.

It must be stressed that the results, shown in tables 10 to 13, are derived from a few pilot experiments only, comprising a very limited number of pigs. It is doubtful, furthermore, whether or not the optimal quantities and the right balance between the different amino acids have been obtained in the experiments. All the results, however, point in the same direction and indicate that a supplementation by certain amino acids of diets low in biological value have a definite influence on the meatiness of pigs.

Influence of Environmental Temperature and Humidity on Carcass Quality

It is a well-known fact that carcass quality is seasonally influenced. Heitman *et al.* (24) found maximum daily gains at 21° C for pigs weighing 90 kilos fed semi ad lib. and decreasing gains at higher and lower temperatures. Otte (40) found increasing daily feed intake when temperatures decreased from 24–20 to 10–8° C. Moustgaard *et al.* (38) reported (table 14) a lower nitrogen deposition at decreasing temperatures and increasing relative humidity. Corresponding with these results the carcass quality was affected, as shown in table 15.

Improvement of carcass quality must, therefore, include better housing, with constant optimal temperatures and relative humidity.

TABLE 13. *Methionine and Lysine Supplementation of a Barley-Maize Diet (Preliminary Results)*

Skim milk, kilos/pig/day	3.0	3.0	3.0	0	0	0
Protein concentrate, g/pig/day*	0	0	0	225	225	225
% barley in cereal mixture	100	40	40	100	40	40
% maize in cereal mixture	0	60	60	0	60	60
Lysine and methionine added to diet			+			+
No. of pigs	19	20	19	17	17	19
Av. thickness of back fat, cm	3.13	3.21	3.13	3.18	3.49	3.44
Slight of lean-measurement, cm	2.77	2.77	2.74	2.59	3.31	2.96
Fat in % of meat in cross section	105	108†	105	103†	137	113

* ⅔ extr. soybean-oil meat and ⅓ meal and bone meal.
† Compared with the results shown in table 8, the figure appears too low. This is probably due to the small number of pigs in these experiments.

TABLE 14. *Protein Deposition in Pigs as Influenced by Climate*

Climate		Ration A		Ration B	
Temp. °C	% rel. hum.	No. of exp.	G N deposited per day	No. of exp.	G N deposited per day
24	90	22	14.37±0.517	10	14.55±1.023
23	50	10	16.50±0.657	6	21.73±1.066
15	70	6	15.73±0.918	6	19.48±0.770
8	70	8	6.53±1.218	8	10.97±1.935
3	70			18	10.73±0.839

TABLE 15. *Bacon Quality of Pigs, Kept Under Different Climatic Conditions*

Air temperature °C	24	23	15	3
Rel. humidity, %	90	50	70	70
Thickness of back fat, cm	3.2±0.07	3.0±0.14	3.0±0.06	3.5±0.13
Thickness of streak, cm	3.4±0.03	3.2±0.08	3.5±0.06	3.6±0.06
Slight of lean-measurement, cm	2.8±0.19	2.8±0.26	2.9±0.15	3.0±0.18
Weight of leaf fat, kilos	1.9±0.09	1.5±0.13	1.5±0.09	2.0±0.11

MEAT QUALITY

Definition of Meat Quality

For many years the main criterion for pork quality has been the proportion of lean meat to fat in the carcass. Quality of pork in the approved sense of meat/fat ratio does not, however, in general incorporate the quality of the meaty part of the carcass. During the last decades meat quality has attracted increasing interest in the production of pigs as well as in the meat packing industry, because consumer preference studies have shown that quality characteristics of pork are important to the consumer.

The problems involved in quality of meat cover extensive fields of research: physiology, nutrition, genetics, bacteriology and technology. However, this section of the paper will be restricted to the significance of the physiological phase of the problem. Thus, many important problems and aspects have to be omitted or briefly mentioned only. The main reason for this is that the authors consider that the physiological aspect of the problem—including some points on nutrition—plays a major part in the numerous factors involved in the quality of fresh pork as well as the quality of industrial meat products from pork.

Evaluation of Meat Quality

It has been known for many years that great variations may occur in the appearance of fresh pork. Even adjacent pork muscles frequently exhibit pronounced variation in colour and general structure. Since Callow (5–8) made the first attempt to define structural variations by differences in electrical resistance, several approaches have been made to define meat quality. However, it must be admitted that objective analytical routine methods, to a great extent, are lacking at present. The evaluation of meat quality is mainly based on subjective tests for colour, consistency, taste and palata-

bility, e.g. the same specifications from which consumers evaluate quality.

Colour and Structure Grading of Carcass

All pigs from the Danish Progeny Testing Stations are graded 24 hours postmortem according to colour and structure of m. long. dorsi immediately after cross section of the carcass at the last rib. The following scores are used (Clausen *et al.* (12)):

Score	Specification
0.5	Gray, same colour as boiled meat. Very moisty surface.
1.0	Very pale, pinkish, moisty surface.
1.5	Pale pink, slightly moisty surface.
2.0	Slightly paler than desirable, almost dry surface.
2.5–3.0	Ideal red colour, dry surface.
3.5–4.0	Slightly darker than desirable, dry surface.
4.5–5.0	Very dark, dry surface.

It will be seen that scores 0.5 to 1.5 in general characterize various degrees of a gray, pale, and pinkish colour and a corresponding more or less moistly structure, whereas the scores above 2.0 indicate variations in red colour and a firm 'dry' structure.

The variations in colour and structure of the fresh pork influence the industrial products, e.g. tinned hams. These exhibit the same colour and structure variations as the fresh hams, from pail-gray, '2-toned' with a 'loose' structure and an impaired palatability to an even red colour distribution with a firm structure and an attractive palatability.

Factors Influencing Meat Quality

Relation between pH and lactic acid formation. Before discussing what is known or thought to be known about the pathways of the physiological mechanism involved in meat quality, it is desirable to point out that there is a close relationship between colour and structure of pork and pH. Thus in gray, pale, pinkish pork with a moisty structure, pH is lower than in pork with a red colour and a firm structure (Ludvigsen (30, 35), Briskey *et al.* (2, 4), Wismer-Pedersen (42)). An important factor in determining the pH level is the formation of lactic acid from the anaerobic breakdown of glycogen.

From table 16 it will be seen that the pH in normal pork is about 6.1 to 6.5 with a corresponding lactic acid concentration of 305 to 752 mg% 30–45 min. post mortem; while in gray, moisty pork, which ranges within scores 0.5–1.0, the pH is 5.3–5.5, and the corresponding lactic acid concentration 1044 to 1201 mg%.

It is well-known that glycogenalysis proceeds post mortem until most of the glycogen present in the musculature is broken down mainly into lactic acid, but probably also into other intermediates in the glycolytic cycle. Usually it is found that the differences in pH and lactic acid in gray, moisty pork and in normal pork, as well as the difference in colour, are less pronounced 12 to 24 hours post mortem. Even pork with a normal red colour and a firm structure 30 min. post mortem may become colourless with a moisty structure in the course of 24 hours post mortem, especially if the glycogen depots are high.

TABLE 16. *pH and Lactic Acid in Skeletal Muscle 30 to 45 Minutes Post Mortem*

	M. Gracilis		M. Psoas		M. Long. Dorsi	
	pH	Lactic acid	pH	Lactic acid	pH	Lactic acid
Normal	6.5 (24)	520 (25)	6.1 (22)	752 (22)	6.4 (24)	305 (24)
Gray, moisty	5.4 (12)	1068 (21)	5.5 (11)	1044 (20)	5.3 (13)	1201 (19)

Lactic acid given in mg %. Number of observations in brackets.

Concomitantly with the increase in lactic acid and the decrease of pH during rigor mortis, important changes in the water balance of the musculature occurs, a condition which generally is termed as a decrease in the water-binding capacity of the musculature.

Various degrees of changes in water balance can be determined by different methods (Grau and Hamm (22), Brisket *et al.* (2), Wismer-Pedersen (42), Judge *et al.* (28)). Using a polar planimeter and recording the ratio of the muscle area to the water area, Briskey *et al.* (2) found that the expressible water ratio increased from 40 minutes to 24 hours post mortem in concordance with the fall in pH. Wismer-Pedersen (42), using the weight loss of the pressed sample to denote the water binding capacity, found that LW (loose water number) increased with decreasing pH and with a significant correlation between LW and pH. Judge *et al.* (28) found that the juice lost upon heating and centrifugation was diminished with increasing pH. Dark, firm loin eye muscles had a greater water holding capacity and a higher pH than lighter coloured, softer muscles.

In sum, it seems justifiable to postulate that the pH level at the time when post mortem lactic acid formation has ceased is a main factor in determining the colour and structure, i.e. the quality of pork. The closer the pH is to the isoelectric point of the muscular proteins, which approximates pH 5.0, the more pronounced is the decrease in the water holding capacity of the muscular proteins followed by an outflux of water containing water soluble cellular components (electrolytes, low molecular peptides and proteins, e.g. myoglobin) into the extracellular space, thus resulting in a moisty and colourless appearance of the meat. The spontaneous outflux of juice from the cells seems to begin at a pH level of about 5.6 to 5.7 and generally reaches maximum at pH 5.2 to 5.3, which seems to be the lowest pH level recorded in pork.

Although lactic acid obviously plays an important part in fixing the final pH level, other factors which may be termed as the buffer capacity of the musculature may be of some significance in the adjustment of the final

pH level. Hitherto the buffer capacity has not been studied in detail, but it seems justifiable to pay some attention to the myoglobin in this respect. Thus the highest pH was recorded in muscles with a high myoglobin concentration (Scaife (41), Briskey *et al.* (4)).

In general, the concept may be expanded towards the conclusion that the final pH level of pork will depend on 2 main factors: lactic acid concentration and the buffer capacity of the musculature. The buffer capacity being low, even moderate amounts of lactic acid may provoke low pH levels of 5.3 to 5.5 with its adverse effects on meat quality, whereas in musculature with a high buffer capacity even reasonable amounts of lactic acid may stabilize the pH at a higher level and the musculature will have a red colour and a firm 'closed' structure. There seems to be quite reliable evidence that the most desirable pH of pork for the meat packing industry should be within the range of 5.8 to 6.0.

Role of muscular activity prior to slaughtering. The role of muscular activity prior to slaughtering considerably influences the quality of pork. Prolonged exhaustive exercise prior to slaughter produced dark muscles with a firm structure and a final pH of 5.89. Various degrees of exercise, both in fasted and fully fed animals, significantly decreased glycogen deposits and, concomitantly with the decrease in muscle glycogen, the pH level rose and the musculature became darker (Briskey *et al.* (2, 3)).

An increased rate of gray-pale musculature with a pH of 5.3 to 5.5 30 minutes post mortem, when pigs are slaughtered immediately after transport to the slaughterhouse, has been reported by Ludvigsen (30), whereas resting of the pigs for 3 hours in the pens of the slaughterhouse prior to slaughtering was followed by a decrease in the incidence of gray, moisty musculature.

Although this may seem conflicting, the discrepancy may be accounted for in recognizable terms. Prolonged exercise is followed by a breakdown of glycogen *in vivo*. Post mortem lactic acid production will be low, the final pH will adjust at a high level, and the musculature will appear dark and firm. On the other hand, relatively brief exercise prior to slaughtering is often followed by an accumulation of lactic acid in the musculature. In such cases (see table 16) the musculature will have a low pH level and appear pale and moisty. This evidence will be discussed further in the following section.

Hormonal regulation of pork quality. Hormonal regulation plays an important role in the physiological processes governing meat quality. Although the primary chemical or physicochemical site of action of the hormones involved remains obscure, the thyroid and the adrenal cortex among other hormone systems seem to play an important part.

POSSIBLE ROLE OF THE THYROID HORMONE. The significance of the thyroid can be demonstrated in 2 ways. Blocking thyroid activity with methylthiouracil the musculature appears pale and moisty (Ludvigsen (30), Briskey (personal communication)). On the other hand, if pigs inclined to have pale, moisty musculature, were fed iodinated casein daily 9 to 14 days prior to slaughtering this resulted in various degrees of red or '2-toned' musculature with a firm structure and a higher pH level than that of the controls (litter mates), the latter having a pale, moisty musculature (Ludvigsen (30, 32)). As the lactic acid concentration in the treated and in the untreated pigs was almost the same, whereas the difference in pH was marked, it has been suggested that the effect of the iodinated casein could not be referred solely to a reduction of the glycogen deposits, but that iodinated casein in some way elicits an effect on the buffer capacity of the musculature (Ludvigsen (34)).

A lowered activity of the thyroid might thus play a role in the pathogeny of the pale, moisty condition of pork. As the thyroid is seasonally varying in activity, it has been suggested that the seasonal variations in the occurrence of pale, moisty pork which is more prevalent in summer than in winter (Ludvigsen (30), Clausen *et al.* (12), Janicki *et al.* (26), Judge *et al.* (27)) are to some extent correlated with the corresponding seasonal variations in thyroid activity.

ROLE OF ADRENAL CORTICAL STEROIDS. Muscular activity in pigs is often followed by a distinct complication of symptoms: dyspnoea, cyanosis, hyperthermia and marked asthenia.

In severe cases the affected pigs often succumb to fatal syncope. In fatal syncope, extremely pale and moisty musculature is an unfailing symptom. Treatment with cortical steroids prior to muscular activity prevents acute complication of symptoms, and it has been shown that the lactic acid produced during muscular activity does not accumulate in the musculature but enters the bloodstream, whereas in the untreated animals the lactic acid accumulates in the musculature and only moderate amounts enter the bloodstream on account of a marked vasoconstriction in the musculature. On the basis of the results it has been suggested that the pale moisty port condition, when visible immediately after death, is a 'stress'-reaction, because the pigs are not able to adapt themselves even to moderate strain of muscular activity. It has further been suggested that pale, moisty musculature is one of the most predominant organic manifestations of 'stress' (Ludvigsen (35), Henry (25)).

POSSIBLE ROLE OF OTHER HORMONE SYSTEMS. It seems to be a well-established fact that the appearance of pale moisty pork is correlated with high meat production (Ludvigsen (33), Clausen *et al.* (12), Judge *et al.* (27)). This has given rise to the assumption that the development of meat producing pigs to some extent might be the result of a gradual change in homeostasis according to a borderline insufficiency of the adaptive hormone systems: the pituitary-thyroid and the pituitary-adrenal cortex axis caused by an increased activity of the growth hormone (STH), which in its general metabolic action is antagonistic to the thyrotrophic hormone (TSH), and the adrenocorticotrophic hormone (ACTH). A few experiments have shown that pale moisty pork may occur after treatment with STH, especially when nitrogen retention is high (Ludvigsen, unpublished).

As pale moisty pork seems to be correlated with high meat production it should be expected that some pig breeds are more susceptible to the condition than other breeds. Judge *et al.* (27), studying 6 different breeds, found that 'light' colour was most predominant in Poland China (60%) and least predominant in Large Whites (16.4%), and firmness described as 'soft' was recorded in 62.5% of the Poland China and 33.6% of the Large Whites. Janicki *et al.* (26) found that pale moisty condition in pork predominates in Large White, where it is less pronounced in the Zlotniki (a crossbred native Polish lard type and Large White).

INFLUENCE OF NUTRITION. Numerous observations indicate that nutrition plays an important role in the pale moisty pork conditions. Lack of protein, as well as vitamins seem to play an important part especially in cases of fatal syncope (Ludvigsen (34)). The nutritional field is a very complicated one in this respect too. However, in general, some attention should be paid to the carbohydrate fraction of the rations used for pigs, as it will appear from the above discussion that the size of the glycogen deposits at the time when the pigs are slaughtered are important to meat quality. High glycogen deposits may cause pale moisty musculature, which indicates that the amount of carbohydrates fed to the animals before slaughtering must be taken into consideration. The problems involved in the matter of meat quality are far from solved. In future investigations considerable attention must be paid to this field of research.

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Quality and quantity of final product—poultry[1]

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THE PROGRESS MADE in the efficiency of production of poultry meat and eggs during the past few years has been pronounced. During the last 30 years the annual egg production in the United States has increased from approximately 120 to almost 200 eggs per hen, an increase of 67%. The feed required per pound of egg is estimated to have been reduced on the average during this period from 5.0 to 3.4 pounds, a reduction of 32%. The annual production of broilers in the United States has grown from 34 million in 1934 to approximately 1.8 billion at the present time. Over 80% of the total chicken meat consumed in the United States comes from commercially reared broilers.

During this period, the time required to produce 3 to 3½ pound broilers has been reduced from approximately 14 weeks to 8 weeks and the amount of feed required per pound of broiler has dropped from 4–4½ pounds to 2–2.4 pounds under commercial conditions. Turkey production has shown a similar reduction of approximately 20% in time required to rear with approximately 30% less feed per pound. These improvements have been brought about by a combination of factors including the development of more productive breeds and strains, better management, housing and disease control, as well as through the application of advances made in nutrition.

NUTRITION—A YOUNG SCIENCE

The prominent role of improved rations in this rapid progress is not surprising since nutrition, as a science, is really still quite young. The vitamin concept did not originate until Sir Frederick Hopkins (England) described the need for an accessory food factor in 1906. The term 'vitamine' was coined by Casimur Funk in 1912, less than 50 years ago, and the following year fat soluble A and water soluble B were recognized as being different nutritional substances. Commercial rearing of confined poultry was not made possible until after McCollum discovered at John's Hopkins University, in 1922, another fat-soluble nutrient, vitamin D. Cod liver oil became widely used in poultry feeds, and the broiler industry came into being.

Following the isolation of thiamine in 1927, studies were continued which led to the isolation of several B-complex vitamins during the 1930's. Of particular importance was the isolation of riboflavin in 1933, since almost all rations for poultry require supplemental fortification with this vitamin. By 1940, pantothenic acid and biotin which prevent dermatitis; niacin, required for growth; vitamin K, needed for blood clotting; choline, needed to prevent perosis; pyridoxine, required for growth; and vitamin E, needed for the prevention of 'crazy chick disease' were known. Further studies led to the isolation of folacin in 1946 and vitamin B_{12} in 1948. Since then, no new vitamins have been shown to be required by poultry; although work on unidentified factors has continued and, at present, evidence exists for 'unidentified growth and hatchability factors.'

During the 30's considerable information was developed about mineral nutrition. The discovery by Cornell workers, in 1936, that only a few parts per million of manganese would prevent perosis (a slipped tendon condition) in growing poultry was of practical importance indeed.

In 1936 it was shown that soybean protein was materially improved in feeding value for poultry by proper heat treatment. This led to extensive production and processing of soybean protein for use in the feeding of poultry. This has contributed greatly to the development of highly efficient rations since properly heat treated soybean protein and corn protein provide an excellent source of amino acids, except for a slight deficiency in methionine. The availability of synthetic DL-methionine, and more recently the methionine hydroxy analogue, as practical supplements, has made the corn-soybean meal supplemented ration a very good and relatively efficient one for poultry.

In 1950, Stockstad and Jukes showed that an antibiotic, now known as chlortetracycline, stimulated chick growth. This brought forth a whole new era of nutritional investigation with the microflora of the host being considered from the standpoint of disease control and effect on nutritional requirements. At the present time, most poultry feeds contain 1 of several antibiotics for growth stimulation and disease control purposes.

The Connecticut group, in 1947, developed a high-energy broiler ration composed of low fiber ingredients. In 1955 importance of energy-protein balance was recognized and the use of stabilized fats resulted in more efficient high energy feeds.

[1] Miscellaneous publication No. 387, contribution No. 3145 of the Maryland Agricultural Experiment Station (Department of Poultry Husbandry).

After all the presently known vitamins had been discovered and made available as supplements, very rapid progress was made in the efficiency of production of high quality poultry meat and eggs. During this earlier period feeds were formulated largely on the basis of critical ingredients. Poultry nutrition reached the scientific age about this time when the formulation of feeds could be done primarily on the basis of the nutritional contributions and cost of ingredients. The specialized chemical industries made available, at relatively low cost, many of the critically limiting nutrients for the fortification of simplified rations composed of cereal grains and soybean meal. At the present time, feed manufacturers add, as supplements to various poultry feeds, the following: riboflavin, pantothenic acid, niacin, vitamin B_{12}, choline chloride, vitamin A, vitamin D_3, vitamin E, vitamin K, calcium, phosphorus, salt, manganese, iodine, iron, copper, cobalt, zinc, DL methionine or the methionine hydroxy analogue, antibiotics, antioxidants, fat, specific drugs (coccidiostats) and sources of 'unidentified growth or hatchability factors.' Lysine is expected to be available soon for similar use. The availability of these supplements at relatively low cost has permitted the formulation of practical poultry feed on a much more flexible basis than was possible in earlier years. In fact, several large manufacturers in the United States and elsewhere are presently using machine calculations for determining the most economical formulas which supply adequate levels of essential nutrients.

RECENT PROGRESS

To illustrate the progress which has been made during recent years, table 1 includes data taken from studies with broilers conducted at the University of Maryland. Since 1952, the time required to produce 3-lb. broilers has been reduced from 10 weeks to 7 or 8 weeks, and the amount of feed required per unit of live weight has been decreased from 2.81 to 1.96, under practical conditions, and has further been reduced as low as 1.04 pounds of feed per pound of broiler, using experimental rations highly fortified with energy and supporting nutrients. The estimated per cent of metabolizable energy retained in the carcass has been increased from approximately 21% in 1952 to 31.8% with some of the most efficient rations. This marked increase in efficiency of broiler meat production is due largely to, *1*) a reduction in the time required to produce a marketable product and, *2*) improvement in the nutritional adequacy, balance and potency of feeds.

TABLE 1. *Comparison of University of Maryland Broiler Results During the Last 8 Years*

Year	Age, Wk.	Av. Body Wt. Lb.	Feed Cons/ Unit Live Wt.	Est. % of Metabolizable Energy Retained in Carcass*
Practical broiler feeds, both sexes				
1952	10	3.08	2.81	21.0
1955	9	3.03	2.37	22.2
1956	8	3.05	1.96	24.4
1960	8	3.51	1.96	26.4
1960	7†	3.09	1.90	
Experimental broiler feeds, males				
1955	7½	3.01	1.60	27.5
1958	6½	3.02	1.19	31.8
1958	7	3.04	1.04	

* 750 Calories of gross energy per lb. assumed. † Males only.

TABLE 2. *Nutrient Requirements of Chickens*[a] *(in Percentage or Amount per Pound of Feed)*

	Starting Chickens 0–8 Weeks	Growing Chickens 8–18 Weeks	Laying Hens	Breeding Hens
Total protein, %	20	16	15	15
Vitamins				
Vitamin A activ. (U.S.P. Units)[b]	1200	1200	2000	2000
Vitamin D_3 (I.C.U.)	90	90	225	225
Vitamine E	see text			
Vitamin K_1, mg	0.24	?	?	?
Thiamine, mg	0.8	?	?	?
Riboflavin, mg	1.3	0.8	1.0	1.7
Pantothenic acid, mg	4.2	4.2	2.1	4.2
Niacin, mg	12	5.0	?	?
Pyridoxine, mg	1.3	?	1.3	1.2
Biotin, mg	0.04	?	?	?
Choline, mg	600	?	?	?
Folacin, mg	0.25	?	0.11	0.16
Vitamin B_{12}, mg	0.004	?	?	0.002
Minerals				
Calcium, %	1.0	1.0	2.25[c]	2.25[c]
Phosphorus, %[d]	0.6	0.6	0.6	0.16
Sodium, %[e]	0.15	0.15	0.15	0.15
Potassium, %	0.2	0.16	?	?
Manganese, mg	25	?	?	15
Iodine, mg	0.5	0.2	0.2	0.5
Magnesium, mg	220	?	?	?
Zinc, mg	20			
Iron, mg	9.0	?	?	?
Copper, mg	0.9	?	?	?

[a] These figures are estimates of requirements and include no margins of safety. Underlined figures are tentative. The protein requirements apply to rations containing 900 Calories of productive energy per pound for starting and growing chickens, and 920 Calories of productive energy per pound for layers. These values are equivalent to approximately 1285 and 1310 Calories of metabolizable energy, respectively. [b] May be vitamin A or provitamin A. [c] The amount of calcium need not be incorporated in the mixed feed, inasmuch as calcium supplements fed free choice are considered as part of the ration. [d] At least 0.45% of the total feed of starting chickens should be inorganic phosphorus. All of the phosphorus of nonplant feed ingredients is considered to be inorganic. Approximately 30% of the phosphorus of plant products is nonphytin phosphorus and may be considered as part of the inorganic phosphorus required. A portion of the phosphorus requirement of growing chickens and laying and breeding hens must also be supplied in inorganic form. For birds in these categories the requirement for inorganic phosphorus is lower and not as well defined as for starting chickens. [e] Equivalent to 0.37% of sodium chloride.

TABLE 3. *Nutrient Requirements of Turkeys*[a] *(in Percentage or Amount per Pound of Feed)*

	Starting Poults 0–8 Weeks	Growing Turkeys 8–16 Weeks	Breeding Turkeys
Total protein, per cent[b]	28	20	15
Vitamins			
Vitamin A activ. (U.S.P.)[c]	2400	2400	2400
Vitamin D_3 (I.C.U.)	400	400	400
Riboflavin, mg	1.7	?	1.5
Pantothenic acid, mg	5.0	?	7.3
Niacin, mg	32	?	?
Choline, mg	850	?	?
Folacin, mg	0.4	?	0.35
Minerals			
Calcium, %	2.0	1.7	2.25[d]
Phosphorus, %[e]	1.0	0.85	0.75
Manganese, mg	25	?	15
Sodium, %[f]	0.15	0.15	0.15
Zinc, mg	25		

[a] These figures are estimates of requirements and include no margins of safety. Underlined figures are tentative. The protein levels shown for turkeys will meet the approximate minimum requirements for early growth in a ration containing 800 Calories of productive energy (1140 metabolizable energy) per pound and for later growth at an energy level of 850 Calories of productive energy (1200 Calories of metabolizable energy) per pound. [b] The protein content of rations for growing turkeys from 16 weeks to market weight may be reduced to 16%. [c] May be vitamin A or pro-vitamin A. [d] This amount of calcium need not be incorporated in the mixed feed inasmuch as calcium supplements fed free choice are considered as part of the ration. [e] At least 0.50% of the total feed of starting poults should be inorganic phosphorus. All of the phosphorus of nonplant-feed ingredients is considered to be inorganic. Approximately 30% of the phosphorus of plant products is nonphytin phosphorus and may be considered as part of the inorganic phosphorus required. Presumably a portion of the requirement of growing and breeding turkeys must also be furnished in inorganic form. [f] Equivalent to 0.37% of sodium chloride.

NRC NUTRIENT REQUIREMENTS

In order to establish guides in the formulation of rations, a subcommittee on poultry nutrition of the National Research Council's (NRC) Committee on Animal Nutrition was appointed in 1943 to prepare nutritional standards for poultry. The first report appeared in 1944 with 3 subsequent revisions. At this time, a fourth revision is nearing completion (2a) and the subcommittee has graciously permitted the inclusion of the NRC recommended nutrient requirements for poultry in this paper. These are shown for chickens and turkeys in tables 2, 3 and 4.

The subcommittee points out in their report that the method of presenting nutrient requirements in terms of concentration of nutrient per unit of weight of ration, though convenient, is basically inaccurate because the nutrient intake of the animal is thereby subject to the absolute level of feed consumption which, in turn, is dependent mainly on the energy concentration of the ration. They further state that the protein and amino acid requirements are particularly subject to this effect because these represent more nearly fixed quantities required per day at a given stage of growth or production, rather than percentages of the ration. For this reason, the subcommittee has indicated caloric levels for which the protein requirements apply and suggests that proportional changes in protein level be made when energy concentrations materially differ from those shown in the footnotes of the tables. The subcommittee points out also that in practical feed formulation it is often desirable to include margins of safety to compensate for possible losses of vitamins during feed processing, transportation and storage, and variations of feed composition and in environment.

ENERGY-PROTEIN BALANCE

The largest part of the feed dollar in the United States is used to buy energy and protein. Accordingly, it is necessary to use these 2 components of the ration most efficiently. By 1952, antioxidants had been shown to serve satisfactorily as stabilizing agents for fats, which made possible the use of feeds considerably higher in total energy concentration. Work at the University of

TABLE 4. *Essential Amino Acid Requirements for Chickens and Turkeys*

Amino Acid	Starting Chicks Ration, %	Starting Poults Ration, %	Laying Chickens Ration, %
Arginine	1.2	1.6	?
Lysine	1.0	1.5	0.50
Histidine	0.3	?	?
Methionine[a]	0.8	0.87	0.53
or			
Methionine	0.45	0.52	0.28
Cystine	0.35	0.35	0.25
Tryptophan	0.2	0.26	0.15
Glycine[b]	1.0	1.0	?
Phenylalanine	1.4	?	?
or			
Phenylalanine[c]	0.7	?	?
Tyrosine	0.7	?	?
Leucine	1.4	?	1.2
Isoleucine	0.6	0.84	0.5
Threonine	0.6	?	0.4
Valine	0.8	?	?
For protein level[d]	20.0	28.0	15.0

[a] Cystine will replace methionine for chicks as long as the ration contains not less than 0.45% methionine. [b] The chick can synthesize glycine, but the synthesis does not proceed at a rate sufficient for maximum growth. [c] Tyrosine will replace phenylalanine for chicks as long as the ration contains not less than 0.7% phenylalanine. [d] The protein requirements apply to rations containing 900 Calories of productive energy per pound for starting and growing chickens and 920 Calories of productive energy per pound for layers. These values are equivalent to approximately 1285 and 1310 Calories of metabolizable energy, respectively. The protein levels shown for turkeys will meet the approximate minimum requirements for early growth in a ration containing 800 Calories of productive energy (1140 metabolizable energy) per pound and for later growth at an energy level of 850 Calories of productive energy (1200 Calories of metabolizable energy) per pound.

TABLE 5. *Suggested Calorie/Protein Ratios for Practical Feed Formulation*

	C/P Ratios*	
	Metabolizable	Productive
Broiler or chick starter (0–5 weeks)	60–65	42–45
Broiler finisher (5–10 weeks)	69–75	48–53
Layers	88–92	60–64
Turkeys (0–6 weeks)	40–44	28–32
Turkeys (6–12 weeks)	55–60	38–42
Turkeys (12–18 weeks)	70–74	48–52
Turkeys (18–24 weeks)	85–90	60–65
Turkey finisher†	100–105	70–72

* C/P ratio is defined as $\frac{\text{Calories per pound}}{\%\ \text{Protein}}$ (see text)

† For fattening.

TABLE 6. *Suggested Minimum Amino Acid/Energy Concentration for Use in Formulation of Broiler Feeds*

Amino Acid	% Amino Acid per 1000 Calories Metabolizable Energy per Pound*		Grams of Amino Acid per Therm of Metabolizable Energy	
	Starters†	Finishers	Starters	Finishers
Methionine	0.31	0.27	1.40	1.20
Methionine + cystine	0.55	0.47	2.50	2.15
Lysine	0.75	0.65	3.40	2.95
Arginine	0.80	0.70	3.65	3.15
Valine	0.60	0.52	2.75	2.35
Tryptophan	0.15	0.13	0.70	0.60
Glycine	0.75	0.65	3.40	2.95

* 50% dehulled soybean meal considered to have 0.7% methionine, 3.25% lysine and 1110 Calories of metabolizable energy per pound. Corn assumed to contain 0.18% methionine, 0.2% lysine and 1600 Calories of metabolizable energy per pound. † Starters to be used for approximately first 4½ weeks.

Maryland showed that as the energy content of the ration was increased, it was necessary also to increase the level of effective protein. Ratios of energy to protein level were suggested for use in formulation of practical rations in order to help maintain the most satisfactory balance of energy and protein for efficient production. These were based on the studies using practical type rations composed largely of corn and soybean meal protein, supplemented with DL-methionine. The term 'Calory protein ratio' (C/P ratio) has been used to designate this relationship between energy and protein and is defined as the Calory content per pound divided by the per cent protein of the feed. Many factors can be expected to influence the C/P ratio most appropriate for economic production; particularly, protein quality. As the protein quality and amino acid balance of the ration are improved, the C/P ratio can be widened. Other factors influencing C/P ratio would include rate of growth, differences in body composition, temperature differences, level of egg production, body size, and other factors which might affect feed intake. C/P ratios, as included in table 5, have been used satisfactorily for the purpose intended. This led to the successful use of rations containing relatively high levels of added fat with no adverse effects. As indicated above, this has been recognized in the recent revision of the NRC nutrient requirements.

Studies by Baldini and Rosenberg (1) have demonstrated a relationship between the methionine requirement and energy content of the ration. This has added greatly to the clarification of the need for supplemental methionine in practical formulas. Studies designed to more nearly determine the requirements for the limiting amino acids in relation to energy would lead to a better means of expressing the requirements. Although protein level or amino acid balance is known to influence the requirements of certain amino acids, the relationships obtained between these amino acids and energy level with corn-soybean meal-type rations should apply quite satisfactorily in practical formulation of rations composed primarily of these 2 ingredients.

Based on studies at the University of Maryland, estimates of the minimum amino acid-energy relationships have been made for certain amino acids for use in the formulation of broiler feeds (table 6). Since either metabolizable or productive energy values may be used, these relationships may be expressed as a function of either energy measurement. The methionine values for corn and soybean meal used in the calculations are given in the footnote. These amino acid-energy concentrations have been satisfactory to support rapid growth of floor pen reared broilers fed practical type rations high in corn and soybean meal.

Unpublished work conducted by Twining (24) Beltsville, Maryland, in collaboration with the University of Maryland, has also yielded information concerning both, the methionine and lysine requirements of growing turkeys by 3-week periods (table 7). These are the 2 most likely to be limiting in turkey rations high in corn and soybean meal.

It is recognized that many other factors besides pro-

TABLE 7. *Experimentally Determined Lysine and Methionine Requirements for White Thompson Poults**

Period Weeks	Lysine†		Methionine†	
	Gms/Therm ME	% Therm ME per lb.	Gms/Therm ME	% Therm ME per lb.
0–3	5.46	1.20	2.03	0.45
3–6	4.68	1.04	1.74	0.38
6–9	4.02	0.88	1.49	0.33
9–12	3.39	0.75	1.26	0.28
12–15	2.95	0.66	1.09	0.24
15–18	2.55	0.57	0.95	0.21

* Unpublished data, P. F. Twining, U.S.D.A., Beltsville, Maryland and G. F. Combs, University of Maryland, College Park, presented at the University of Maryland 1960 Nutrition Conference for Feed Manufacturers, March 17, 1960. † 0.7% methionine and 3.25% lysine used for 50% protein soybean meal.

tein level and amino acid balance will influence the optimal amino acid-energy proportion, such as differences in growth rate in relation to body size, activity, temperature, level of egg production, and size of hen. In fact, any factor which will modify the animal's need for energy in a manner not quantitatively proportional to the effect on the requirements for amino acids, and vice versa, should lead to slightly different optimal amino acid-energy relationships. Much work will be required to more nearly quantitate the effects of these several factors so that the requirements for these nutrients may be more nearly predicted for specific conditions.

Ideally, the requirements should be calculated for each amino acid and for energy from equations including appropriate terms for each of these factors. Such has been attempted in estimating the methionine requirement for layers as given below.

AMINO ACID REQUIREMENTS OF LAYING HENS

Differences in level of egg production, egg weight, body size, temperature, activity level, feed potency, as well as protein quality and amino acid balance, lead to marked variance in the apparent requirement for protein by the laying hen.

Results obtained with laying hens at the University of Maryland in 1958 and 1959 have permitted the partition of the requirement for methionine into maintenance, change in body weight and egg production functions (6). This is set forth in *equation 1* below. Previous work conducted at Maryland in 1941, by Byerly (4), provided an equation for the calculation of the feed requirement of laying hens considering maintenance, change in body weight, and weight of egg product. The Byerly equation has been modified to yield Calories of metabolizable energy rather than weight of feed required (*eq. 2*).

1) $M = 0.05W \pm 6.2\Delta W + 5E$

and

2) $C = T\,(1.516W^{0.653} \pm 3.264\Delta W + 3.29E)$

Where M = daily methionine requirement per hen in milligrams

C = daily requirement for metabolizable Calories per hen

W = average body weight per hen in grams

ΔW = average daily change in body weight per hen in grams

E = average grams of egg product per hen per day

and T = approximate temperature correction factor for each season.

With these 2 equations, one can calculate the requirements for both, methionine and Calories of metabolizable energy per hen per day for varying conditions. The temperature factor which has also been included in the modification of Byerly's equation (*eq. 2*) is based on the data obtained by Hill (17) in which he has obtained average relative efficiencies of feeds during different seasons in relation to those predicted by the Byerly equation. To correct for temperature in a general way by season (equivalent to temperatures in Ithaca, New York), the following values are given for T in *equation 2* above: 0.98 for fall; 1.05 for winter; 1.0 for spring; and 0.94 for summer.

The grams of methionine required per therm of metabolizable energy may then be calculated by merely dividing M by C as determined in *equation 1* and *2* above. This can be converted into per cent methionine for a specific ration of known energy content.

After the methionine requirement is determined for specific conditions, one may approximate the levels of the other essential amino acids required by applying conversion factors based on the relative amounts of the amino acids present in egg protein. This approach has been used by Fisher (15). Using the values of Block and Weise, the following conversion factors are multiplied directly by the calculated requirement for methionine: methionine, 1.0; methionine plus cystine, 1.7; histidine, 0.73; isoleucine, 2.09; leucine, 2.85; lysine, 2.09; phenylalanine, 1.76; phenylalanine plus tyrosine, 3.0; tryptophane, 0.49; threonine, 1.52; and valine, 2.24.

Such an approach recognizes certain of the many factors which do influence the requirement of the animal for energy and for protein independent of each other. Since feed intake is largely dependent upon energy level of the ration, and because both energy and protein constitute the largest part of the feed cost, the need for such improved approaches to the estimation of the protein and amino acid requirements for poultry is obvious.

C/P RATIO AND BODY COMPOSITION

In addition to energy concentration, the protein adequacy of the ration also has been shown to influence feed caloric intake. In work at the University of Maryland (8, 12, 13), isocaloric rations containing different levels of protein and isonitrogenous rations containing different levels of energy were used to provide a range of productive Calorie-protein ratios ranging from 35 to 70:1. All rations had sufficient energy density to insure that capacity would not limit feed consumption. As the C/P ratio was widened, a 2½-fold increase in the body fat content (from 5.6% to 14.2%) was observed with relatively little difference in rate of gain. There was no effect on rate of gain when the C/P ratio was widened from 35 to 49, despite the fact that 68% more fat was present in the carcass of chicks fed the C/P 49 rations than was present when the C/P 35 ration was used. By merely reducing the level of protein without altering its amino acid quality, the body fat content was increased from 5.6 to 9.4%. The energy content of the carcass also was varied from 770 to 930 Calories per pound before differences were observed in rate of gain. Further increases in carcass energy and fat content and decreases in moisture content were observed with ration having wider C/P ratios.

The positive correlation between C/P ratio and body

fat content indicates that the chick is able to increase appreciably its energy intake in an effort to obtain sufficient protein. In this way, the growing chick can overcome, to some extent, 'protein deficiency stress' as measured by rate of gain, although the increase in energy intake is reflected in higher fat content of the carcass. If isocaloric rations are used, this overconsumption of energy results in poorer feed conversions.

The use of rate of gain as the only measure of response in studies of protein and amino acid requirements, without determining changes in body composition, makes it difficult to interpret results properly. Since lowering the level of protein in relation to energy content causes a relative overconsumption of energy, the minimum protein or amino acid level which will support optimal rate of gain can be considered only as that level below which the 'protein deficiency stress' can no longer be overcome completely, and not necessarily the level required for an optimal nutritional state. It has been shown also that the percentage of dietary protein retained in the carcass is influenced greatly by the energy intake of the chick (19).

FATS AND PELLETING

Upgrading the energy content of rations for broilers by the inclusion of fats has been shown to stimulate rate of gain and reduce the amount of feed required per unit of product (10, 14). Levels as high as 20% can be readily used without difficulty, and in some special studies at Maryland 50% of the total feed for broilers was composed of fat (9).

The physical form of the feed has been observed to affect the growth response of broilers to added fat. In several experiments, involving practical broiler feeds in mash or pelleted form, the inclusion of approximately 10% fat in mash feeds resulted in consistent and statistically significant improvements in average body weights. However, when the same feeds were pelleted, no improvement in growth was noted from the addition of fat (10).

Pelleting of the low fat feeds also improved the growth rate, but only with the feeds without the high level of added fat. The low fat feeds contained approximately 1000 Calories of productive energy per pound.

It would appear that the growth promoting value of pelleting and of added fat results primarily from an increase in potency of the feed and a subsequent increase in nutrient consumption. Studies from this laboratory involving paired feeding of corn oil and corn starch containing rations have failed to show any differences in the results. With ad libitum feeding of mash diets, however, inclusion of various fats have resulted in improvement in growth.

Studies by the Cornell group (5) and others (7, 20) have shown that unextracted full-fat soybeans can be heat treated and rendered satisfactory for use in poultry feeds.

OTHER DEVELOPMENTS

It is not possible to cover many of the recent developments in this paper. However, a few are included.

It has been shown that the feeding of certain enzymes to rations containing high levels of barley, or the soaking of barley prior to feeding, results in improved digestibility, better growth and feed conversion. Recent studies suggest that the calcium and phosphorus levels used for growing turkeys and broilers may be reduced slightly. Work with laying hens suggests the phosphorus level may be dropped to approximately .4 to .45%, but the calcium level should be increased. With high level production and good feed conversions, calcium levels as high as 5 to 6% of the total diet have been used in all mash feeds without adverse effects.

The potentiation of certain antibiotics, particularly the tetracyclines, has been shown to occur by reducing the level of calcium in the ration. Levels as low as 0.5 to 0.7% calcium appear to be tolerated in young growing broilers during periods of high level antibiotic feeding without adverse effects on bone ash, growth, or feed conversion. This results in approximately a twofold increase in the blood level of the antibiotic during periods of high level feeding.

Recent studies with antibiotics have revealed growth stimulating properties of comparable magnitude with those obtained earlier (16). Marked reduction in the requirement for potassium by the turkey poult has been demonstrated in our laboratory (22) as a result of antibiotic administration, supporting the concept that antibiotics improve the absorption of nutrients.

Zinc supplementation of rations for turkeys and chickens has been shown to be of practical importance. Work in our laboratory indicates that the growing turkey requires at least 50 parts of zinc per million of ration during the first few weeks for proper growth and approximately 70 parts per million for proper bone formation and feather formation (23). Zinc also has been shown to be needed for hatchability and egg shell formation (2). The demonstration by the Texas A & M group that molybdenum is essential for poultry (18) and the finding that selenium is required at low levels under certain conditions (21) are noteworthy.

Evidence still exists for organic and inorganic factors, not at present recognized, capable of stimulating growth and hatchability. The isolation of crystals which are effective in growth promotion of chicks and turkeys has been reported by Couch (11). In studies at Maryland with simplified practical rations, the addition of animal protein supplements has resulted in rather consistent responses in growth of approximately 2% to 8 weeks with broilers. No differences have been observed in feed conversion. Similar results have been obtained with turkeys. Rations composed of corn, soybean meal, and alfalfa meal plus supplemental minerals and vitamins also have maintained high egg production with laying hens.

SUMMARY

It appears clear that the rapid accumulation of research information followed by equally rapid application in the formulation of practical feeds has led to marked improvements in efficiency of production of poultry meat and eggs. Due to the availability of vitamins, minerals, and certain amino acids and drugs, the formulation of practical feeds for poultry can be based almost entirely upon nutritional contributions of various ingredients in relation to cost, through a scientific approach to meeting the requirements for specific purposes under defined conditions. The importance of nutrient potency, and amino acid adequacy of rations, has been emphasized because of their influence of relative efficiency of production. The rapid progress which has been made in the past should alert one to opportunities which lie ahead and accomplishments of the past few years can scarcely be more than a reflection of that which is to come.

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Panel Summary—Animal Nutrition and Food Production

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I THINK THAT PROBABLY THIS PANEL has had the most difficult task of any, because we have had to range over such a fantastically wide field. Many of you whom I see here are interested in only one small aspect of this whole animal economy; not all of us are necessarily interested, except indirectly, in the nutrition of man. But here this morning we have attempted, or should have attempted, to try to effect this very complex piece of integration.

We have ranged over a very wide field, from the more practical details of husbandry to questions of age changes in organoleptic perception, and without reviewing in detail the individual questions there are several points which can be made.

The first point is that our knowledge is still woefully imprecise. We do not know whether acceptance of approximations such as the TDN system as a measure of the energy value of feeds results in very great error in farm practice, we do not know the extent of area deficiencies of essential trace elements, we do not realise the full implications of longevity in cattle in relation to particular economic circumstances, nor can we, in fact, do more than make most general recommendations regarding protein levels and protein quality for swine. This lack of precision in our knowledge is of considerable importance when it comes to application of our findings to the underdeveloped countries.

This leads to the second general point that almost all of the knowledge which we have discussed this morning is knowledge which has accrued in the well-developed countries of the world. For example, the prodigious growth of the poultry industry in the United States, is, to my mind, one of the most amazing pieces of integrated work that has ever been done. To have taken an industry, as the people in America have done, and to build it up to such a peak of efficiency and perfection in such a relatively short time amazes people who live in other countries. This, of course, has been due almost entirely to the efforts of workers in the United States.

Our real problem in this symposium is to try to apply this and kindred knowledge which the Western world and also the Antipodes have acquired, to the underdeveloped countries. Here there are barriers for us no less than there are barriers for those unfortunate people who do not everyday get sufficient to eat. These barriers for us, stem from the fact that we are, ourselves, dealing in isolated economic cultural and physical environments.

The poultry industry, I mentioned earlier, has been largely built up on a corn soy-bean dietary. To that dietary to obtain best results one has to add, at the present time, about 24 supplements, in the form of minerals, vitamins and medicaments. The problems of dealing with an economy which is based on cassava is not the simple direct application of this type of detailed knowledge. Rather we have to start again from the very beginning; we have to approach these problems very humbly and try to apply scientific method to them just as we have applied scientific method to the problems in our own countries. Dr. Cuthbertson, in his opening remarks, mentioned that half the land of this globe is in tropical areas and he also pointed out the amazing potential that those countries have in terms of the production of grass, the production of grain and the production of animals for human food. The problem is, how do we realise these potentials?

Besides this air of humility with which I suggest we approach these general problems of the underdeveloped countries, what we must do is to ensure that the peoples of these countries are educated. Educated in terms not only of nutritional knowledge, but educated in all that modern living involves. Professor Bigwood pointed out that in the days of colonial administration under that benevolent despotism which my own and other countries indulged, we could more or less dictate nutritional policy in the agricultural field. That era has gone; it is not coming back.

The only way which remains to increase the welfare of these communities is by the adoption of a well-balanced educational policy. We can help these communities by helping them to plan; we can help them by suggestions, we can help through FAO and allied organizations as has already been suggested in a question to one of our panel members. In doing so, however, we have got to remember that we are dealing with different sets of conditions, different soils, different climates, extreme dispersion of the population in some instances, and an over-all and overwhelming lack of technical knowledge. Education in the elements of nutrition, application of the scientific principles which are involved in nutrition, education in agricultural methods, and education in animal husbandry, is the only lasting way in which we can make progress for these very unfortunate people.

PANEL VII

Three Hours Around the World—New Possibilities in Nutrition Research

Chairman: W. W. Griffith, USA
Moderator: V. Ramalingaswami, India

Nutritional aspects of calculus disease of the urinary tract

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As the physicists say, there are two modes. . . .these are the opposites; in general, they are hyperxis (excess) and ellepsis (deficiency, failure). ARISTOTLE, *Physics* (35)

DEFICIENCY AND EXCESS constitute the basic pathogenetic mechanisms in nutritional disease. Calculus formation in the urinary tract has been related either to a deficiency of a substance(s) which in normal urine aids in keeping the crystalloids in a soluble state or to the presence of a substance(s) which enhances the precipitation of crystalloids. In his Ramon Guiteras lecture delivered in 1934, Joly concluded a masterly analysis of the aetiology of stone by endorsing the deficiency disease hypothesis as the most "plausable and probable" which explained all the then-known principal features of the condition (18). He believed that 'vitamin starvation' acted primarily on the renal epithelium and through it on the colloid mechanism of the urine. In the intervening quarter of a century, although much new knowledge has accrued, the deficiency disease hypothesis has not made much headway and the aetiology and pathogenesis of calculus formation remain unclear. In this review, we shall attempt to survey the field with special reference to malnutrition and in doing so we have to necessarily tread on 'non-nutritional' territories. Our concern will be with mechanisms rather than therapeutics.

Bladder stone disease will receive particular emphasis. As a distinct clinical entity occurring with predilection among children, it has long been recognized. Once common in the countries of the West, its incidence there has declined remarkably with the rise in living standards (18) and the triad of endemicity, lower urinary tract localization and urate composition is no longer in evidence (25). However, it continues to be an important health hazard to children in several countries in South East Asia (30). Like goitre and fluorosis, it occurs in an endemic form in certain geographic areas. Malnutrition, especially vitamin A deficiency, has been advocated strongly as a major aetiological factor on the basis of animal experiments, but in human disease authentic evidence in its favour is as yet scarce. Some observations made by us on children with bladder stone disease in the Delhi area will be presented.

The subject will be discussed under three headings: *1*) urinary calculi in general. This will deal for the most part with current concepts regarding factors leading to stone formation and with upper urinary tract stones, which constitute the bulk of urinary stones in western population; *2*) vesical calculi in general; and *3*) vesical calculi in the Delhi area.

URINARY CALCULI IN GENERAL

Many excellent reviews of the mechanism of stone formation have been published recently (16, 21, 39). The problem is one of minerals and matrix and their interaction in the microenvironment of the urinary tract. All stones are believed to be composed of an organic matrix (2.5 %) and an inorganic crystalline body (97.5 %) (4), but more of these factors presently. The microenvironment is an ill-defined entity influenced by a variety of forces, but one of its attributes, the urinary pH, is known to exert an important influence on stone formation. Calcium phosphate and magnesium-ammonium phosphate precipitate out in an alkaline medium; hence the occurrence of these salts in stones of patients with urinary tract infections with urea-splitting organisms. Oxalates of calcium, on the other hand, precipitate over a wide range of pH. Cystine stones in cystinuria and oxalate stones in primary oxalosis are entities aetiologically related to inborn errors of metabolism and need not be considered further.

The minerals. The common chemical ingredients of calculi are calcium, magnesium, ammonium, oxalate, phosphate and uric acid (32). Calcium oxalate and phosphate either singly or in combination account for the greater part of the mineral matter (31). Petrographic crystallography and quantitative chemical estimation in a recent unselected series of 287 stones from the South-eastern part of the USA reveals calcium oxalate as the major mineral component, calcium phosphate being next in order of frequency and being more frequently encountered in females than in males (17). There are no doubt variations from area to area in the precise

chemical composition of calculi and, as will be evident subsequently, uric acid relatively so inconspicuous in the renal stones of the West becomes a major component of bladder stones.

In view of the pivotal position held by calcium—the bulk of cation in most stones—it is hardly surprising that the role of hypercalciuria in calculus formation has received a great deal of attention. That this is an important factor in those cases of renal stone associated with hyperparathyroidism, renal tubular acidosis, bone disease (multiple myeloma, metastatic carcinoma), prolonged immobilisation, hypervitaminosis D, milk-alkali syndrome and idiopathic hypercalciuria cannot be questioned. Evidence has been presented recently to show that in dogs hypercalcaemia and by inference hypercalciuria, even of short duration, say 24 hours, produced by injection of paratharmone, leads to structural alterations in the distal nephron, consisting of epithelial degeneration, intratubular calcification and obstruction (8). But alas, in spite of all this, no crucial villain-of-the-piece role can be assigned to hypercalciuria. Hypercalciuria can occur without calculi as in Fanconi syndrome and calculi can and frequently do form in the absence of hypercalciuria. Indeed a large proportion of cases of renal stone are not accountable on the basis of hypercalciuria either 'idiopathic' or of known cause. Approximately two thirds of the patients in a well-worked up British series had average urine calcium values either within or below the normal range (15). In Albright's clinic, no more than one half of the cases of renal stone can be attributed to the factors mentioned above (23).

Unlike calcium, variations in oxalate excretion seem to be totally unrelated to calculus disease (9, 22). The role of increased excretion of inorganic phosphate is not very clear but, on present evidence, it does not appear to be an important factor (15). The most common cause of uric acid stones of renal origin would appear to be a persistently acid urine rather than the excretion of excessive quantities of uric acid (13).

In a discussion of the mineral factor, it is necessary to consider not only the super-saturation of urine with calcium salts but also the factors influencing the solubility of these salts in the urine. The solubility of calcium oxalate is reported to be increased in the presence of magnesium (12). It has been suggested that citrate in the urine chelates calcium and holds it in solution even in an acid pH (37) although recent studies (41) indicate that at physiological levels the effects of citrate are minor. Colloidal substances in urine are claimed to exert a protective influence against stone formation by maintaining calcium in solution (7). Glucuronides are known to form soluble calcium complexes and it is suggested that they may exert a protective influence (33). Amino acids increase the solubility of sparingly soluble salts such as calcium and magnesium phosphates and it has been reported that 42 % of stone patients excrete reduced amounts of amino acids in urine (20).

The matrix. The numerous lacunae in the hypotheses attempting to explain calculus formation purely in terms of alterations in the concentration of minerals and their solubilisers in urine have encouraged investigators to turn their attention to the stone matrix. The matrix, composed of mucoprotein and mucopolysaccharide, was conceived not merely as an incidental cement substance but also as one capable of extracting mineral substances from solution, thus playing a primary pathogenetic role. There is already considerable evidence to indicate that acid mucopolysaccharides of cartilage may act as 'target substances' for the deposition of calcium salts (36). It has been observed that calcification of rachitic cartilage under the influence of vitamin D is accompanied by histochemical alterations in the ground substance of cartilage, presumably in the mucopolysaccharide (34).

Boyce and his group demonstrated increased excretion of mucoprotein in urine in patients with renal stone and the mucoprotein was shown to combine with calcium with avidity *in vitro* (5). The stone matrix has been shown to consist of a combination of mucoprotein and acid mucopolysaccharide by micro-histochemical procedures (6). Baker succeeded in showing that alterations in renal tubule connective tissue mucoprotein immediately preceded calcification in a variety of experiments designed to produce renal calculi (3). Renal biopsy studies in cases of renal stone revealed bilateral alterations in renal tubular stromal matrix (2). Murphy (24) finds 'colloid' in all renal stones which he classified into types I and II. In the type I stone the colloid presumably plays a dominant part while in type II, it merely plays a passive role of filling the interstices. It is interesting that urinary excretion of calcium is higher in patients with stones of type II than in those with stones of type I.

It is thus clear that the matrix substance(s) must receive consideration in any pathogenetic schema of urinary calculus and the future must inevitably lie in a further exploration of the nature and source of mucoproteins and mucopolysaccharides of the stone matrix.

Nutrition and renal calculi. Reference has been made to the stout defence put up by Joly (1934) in favour of nutritional deficiency as the most probable cause of urinary calculus. The experimental production of urinary calculi in rats by the use of diets deficient in vitamin A formed the bed-rock of the nutritional hypothesis (14, 29, 38). It was believed that the keratinised metaplastic epithelium of the urinary tract in vitamin A deficiency formed a nidus for stone formation. The epidemic wave of oxalate stones that occurred in Central Europe after World War I was never adequately explained but was generally attributed to nutritional deficiency (11).

Into this rather static scene, interest was injected by the recent demonstration of oxalate nephrocalcinosis in young cats in pyridoxine deficiency (10) and of renal calculus formation, with apical renal papillary concretions, in white rats fed on a diet deficient in pyridoxine and rich in glycine (1). The calculi in rats seemed to resemble the oxalate calculi seen in man. It remains to

be explored whether a human counterpart of this phenomenon occurs.

VESICAL CALCULI IN GENERAL

The aetiology of bladder stone is enveloped in even greater mystery than that of renal stone. It is not known whether the factors that influence the formation of renal stone would be applicable to bladder stone. Infection and stasis are common denominators but the association with metabolic hypercalciuria so conspicuous in some cases of renal stone is very inconspicuous in cases of bladder stone. Niebel (27) states that whereas over 700 proved cases of hyperparathyroidism with calcification in the kidney and ureter have been reported in the world literature, only 2 cases of primary calculi of the bladder with hyperparathyroidism have been brought to light so far. Niebel added the third case.

In discussing this subject, tribute must be paid to the memory of Sir Robert McCarrison whose demise, recently announced, the world of medicine and nutrition deeply mourns. McCarrison, the brilliant experimental physician, produced bladder stones in albino rats by feeding them diets deficient in vitamin A and containing excess of lime. He showed that cereal foods ranged themselves in the following descending order of stone-producing potency: whole-wheat flour, oat meal, north Indian millet (Bajra), white flour, rice and south Indian millet (Cambu). Summarising his work in an address to the Royal College of Surgeons of England, McCarrison (19) stated: "In India, stone is a poor man's disease. . . . Taking these factors into consideration with those I have already presented, the conclusion seems to me to be irresistable that in India, urinary calculus is a disease of faulty nutrition and that the same food faults which produce the malady in rats produce it also in the people of India."

Since the days of McCarrison, no further evidence has been adduced to prove the deficiency disease hypothesis. It is known that the chemical composition of the experimentally produced stone in rats is different from that of stone in man (26). Furthermore, the widespread distri-

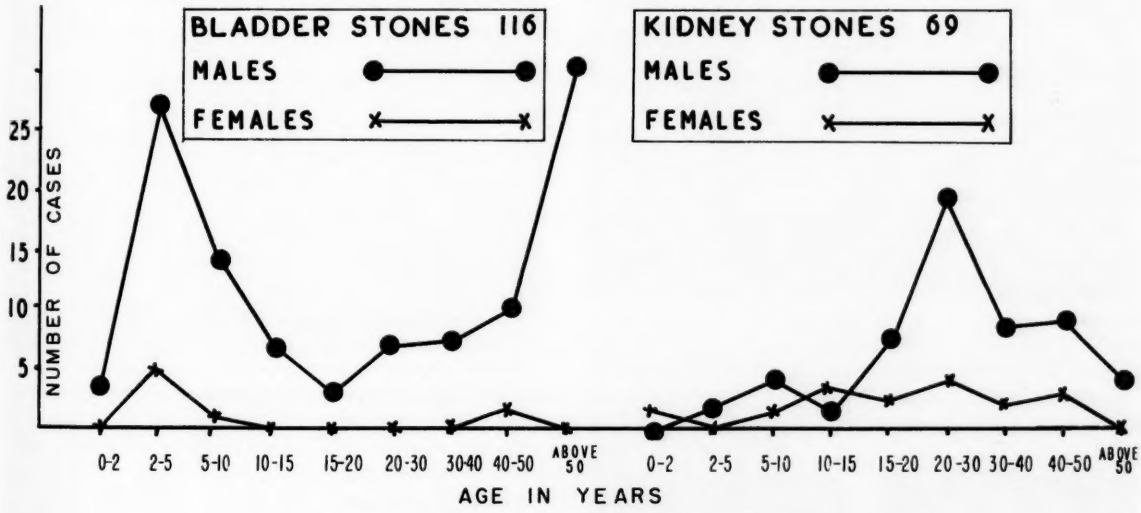

FIG 1. Age and distribution of cases of urinary calculi

TABLE 1. *Values of Serum Plasma Constituents in 22 Cases with Bladder Stone and in 10 Controls*

	Calcium (mg/100 ml)	Phosphorus (mg/100 ml)	Alkaline phosphatase (K.A. Units/100 ml)	Citric Acid (Ug/ml)	Seromucoids (mg/100 ml)
Mean values in patients	9.83	5.3	8.54	25.35	17.25
S.D.	±0.897	±1.177	±2.559	±6.559	±7.62
Mean values in controls	9.25	4.29	6.694	26.73	11.87
S.D.	±0.716	±1.103	±3.225	±3.01	±2.75
Value of t	1.7935	2.2872	1.7251	0.629	2.1544
Value of P	0.05 < P < 0.1	0.02 < P < 0.05	0.05 < P < 0.1	0.5 < P < 0.6	0.2 < P < 0.05

t refers to Fisher's t-test and P to probability.

bution of deficiency disease throughout Southeast Asia contrasts with the restricted foci of bladder stone and makes direct causal relationship unlikely. Severe clinical vitamin A deficiency occurs with greater frequency in parts of South India than in North India, and yet there (South India) bladder stone is decidedly less frequent. Oomen (28), whose experience with clinical vitamin A deficiency is unrivalled, does not find a direct relationship between bladder stone and vitamin A deficiency. It is little wonder therefore that Passmore (30), in a report on the epidemiology of bladder stone disease in Thailand, was forced to conclude that stone formation in the bladder is probably due to a disturbance of a complicated biochemical mechanism, the nature of which is unknown.

VESICAL CALCULI IN THE DELHI AREA

Bladder stone disease occurs with considerable frequency among the children of Delhi and its environs. In the Safdarjang Hospital, Delhi, a total of 185 cases with urinary calculus was admitted for surgery during the years 1955 and 1956 and out of these 116 were bladder stones. The age and sex distribution of these cases is presented in figure 1.

For the present study, 22 unselected cases with bladder stone under the age of 10 years were chosen. A group of 10 children of similar age group with no bladder stone and clinical disease was studied in a similar manner as controls.

The investigations included: clinical appraisal with special reference to dietary and nutritional status, serum calcium, phosphorus and alkaline phosphatase, citrate and mucoprotein levels of the blood, serum proteins, sodium and potassium, histological and histochemical study of matrix of the stones, histological study of the wall of the urinary bladder. A study of pH and bacteriology of urine was routinely made. In 5 children with bladder stone, a calcium balance study was made.

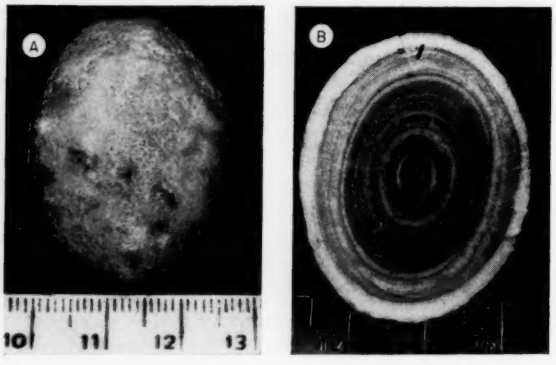

FIG. 2. A: Macroscopic appearance of the external surface of a typical type B stone. It shows fine nodules, few short spikes and patches of smooth surface. × 2.5. B: Cut surface of the stone shown in figure 2A. Large number of regular laminations enclosing a centrally located nucleus are seen. × 2.5.

Details of techniques and results are being prepared for publication elsewhere (Aurora and Ramalingaswami 1960). A resume of the more pertinent findings is presented here.

TABLE 2. *Dietary and Urine Calcium Values in Children with Bladder Stone*

Age (yrs), and sex of subjects	Daily calcium intake, mg.	Daily urinary calcium excretion, mg.
5, M.	1028.9	82.8
8, M.	1016.8	189.2
3, M.	1010.4	8.14
2½, M.	1021.7	26.6
4, M.	1235.4	83.9

Figure 1 reveals the marked preponderance of bladder stone disease in male children. The condition is prevalent in the poorer classes and even allowing for the fact that the bulk of the hospital patients is constituted by these classes, it seems that there is some relationship with poor economic status.

The diets of the affected children were no different qualitatively and quantitatively from those generally consumed in this part of the country by the poorer sections. Clinical evidence of vitamin A deficiency was lacking.

Table 1 shows the concentration of serum constituents. The only significant feature is the elevation of serum mucoprotein level in cases with bladder stone as compared with the controls. The mucoprotein was estimated according to the method of Weimer and Moshin (40). The serum calcium and alkaline phosphatase levels are not significantly different from the controls and so are the levels of serum proteins, citric acid, sodium and potassium.

Table 2 gives the dietary intake and urinary excretion of calcium in 5 children with bladder stone. The children were acclimatised to a diet containing approximately 1000 mg of calcium for a minimum period of 7 days before being studied. Collections were made for 3 consecutive days and the values represent the daily averages. A wide variation in urinary excretion of calcium is noticeable and even on such a high intake as 1000 mg, the urinary excretion in all cases was less than 200 mg, thus indicating that hypercalciuria could not be a factor.

Qualitative chemical analysis revealed that all stones contained calcium, oxalate, phosphate and uric acid. On the basis of the gross morphology of the external and cut surfaces, the stones could be divided into several types, the commonest type (B) is illustrated in figure 2.

Sections of stone matrix prepared according to the method described by Boyce and Sulkin (6) were stained by a variety of histochemical procedures. A gelatinous matrix was present in variable quantity in all stones, both in the nucleus and the body of the stone. In the nucleus, it was amorphous; and in the body, it was fibrillary with amorphous matrix cementing the fibrils

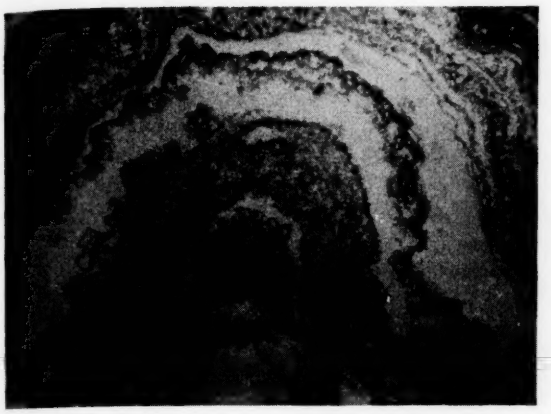

FIG. 3. Section of a stone stained with haematoxylin and eosin. It shows a single nucleus composed of amorphous type of matrix surrounded by a few laminations. × 22.

FIG. 4: Peripheral portion of the section shown in figure 3. Besides the large number of laminations a part of the nucleus is also seen. × 110.

(figs. 3 and 4). Two types of mucopolysaccharide could be demonstrated in the matrix—one which may be termed 'neutral', giving a positive reaction to periodic acid—Schiff alone and the other 'acid', giving a positive reaction with alcian blue and Rinehart-Abul-Haj staining procedures and a metachromatic reaction to toluidine blue.

Another feature was the presence of well-formed cellular elements in the matrix of the stones, predominantly located in the body; the cells comprised of epithelial elements, polymorphs and occasional mononuclears (fig. 5). Weigert stain revealed the presence of a large number of gram-positive cocci in most stones. The bladder biopsies revealed chronic inflammatory reaction in the submucosa together with focal hyperplasia of the lining epithelium (fig. 6). There was no keratinising metaplasia.

Summing up the results, it may be stated that the study has raised innumerable questions and has not identified any specific aetiological factor. There is probably a constellation of factors involved in the life history of bladder stone disease which, in varying combinations, influence the disease in different geographic areas. The presence of mucopolysaccharide in the matrix of all stones is believed to be of significance. The presence of cellular elements and microorganisms in the matrix is likewise believed to be of associational significance. How these interact in the microenvironment of the urinary tract and their relation to stone formation are yet to be clarified.

SUMMARY AND CONCLUSIONS

In the earlier part of this presentation, current concepts concerning the aetiology and pathogenesis of urinary

FIG. 5: Section of stone stained with Feulgen stain. It shows large number of cells entrapped in between the lamellae. × 375.

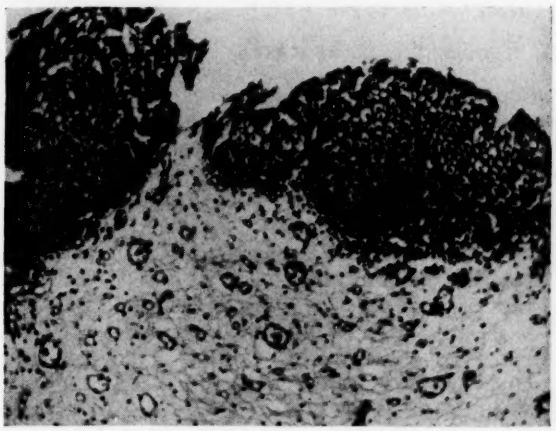

FIG. 6: Section of a bladder biopsy stained with haematoxylin and eosin. It reveals focal hyperplasia of the mucosa, the cells of which are vacuolated. The submucosa is oedematous, congested and infiltrated with inflammatory cells. × 110.

calculus disease have been presented in a general way. It was surmised that despite an early start, the hypothesis based on nutritional deficiency, particularly on vitamin A deficiency, has not made much progress in recent years.

In the later part, attention was focussed on bladder stone disease which occurs in an endemic fashion in certain circumscribed areas of the world and predominantly in young male children. Its aetiology is even more obscure than that of renal calculus. Here too, it was concluded that adequate proof of a pathogenetic relation with nutritional deficiency has not been forthcoming.

Towards the end, a summary of pertinent observations made by us in a study of bladder stone disease among the children in Delhi area is presented. Mucoprotein, cellular elements and microorganisms have been demonstrated in the matrix of the stones. Elevation of serum mucoprotein has been observed. How these findings are related to one another and fit into the jig-saw puzzle of bladder stone disease is for the future to unravel.

We would like to record our appreciation of the help given us by Col. R. D. Iyer (Safdarjang Hospital), Dr. P. D. Gaitonde (Irwin Hospital), and Dr. E. J. Lazaro (Institute Hospital).

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The classical deficiency diseases

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THE CLASSICAL DEFICIENCY DISEASES, named in the order of their prevalence among world populations, are beriberi, pellagra, scurvy, rickets and xerophthalmia. To these should be added ariboflavinosis, which has been recognized only within the past four decades and in this sense does not fall into the category of 'classical' disease. Its symptoms are always mild to the best of our knowledge and it has never caused deaths of human beings so far as we know. In prevalence, however, it ranks very high as judged by recent surveys (5, 7), chiefly military, in Asia; and our experience with the disease in experimental animals suggests that its consequences in humans may be far more severe than we are able to recognize. In some rice-eating countries such as Formosa and Korea it is far more prevalent than beriberi or any other classical nutritional deficiency.

The great fatal epidemics of beriberi, pellagra, scurvy and rickets are now things of the past for various reasons. The most important reason for their disappearance is of course that their causes and remedies for them are known and available wherever they are brought to the attention of physicians. What concerns us is the continuing widespread occurrence of these diseases in a form mild enough to make their diagnosis difficult (6). To any one who has lived in Asia, Africa or Latin America it is a familiar fact that the masses in general live and die without the advice of a qualified physician. Under these circumstances the classical deficiencies provide an undercurrent of disease which may constitute a great menace to health and a serious deterrent to productivity, happiness and the capacity to learn. What we need are measures which can be applied en masse to abolish these diseases, without detailed study and treatment of individuals, a task that is too gigantic to contemplate.

Which of these classical diseases remain as serious menaces to health and welfare, in spite of the disappearance of the great epidemics? Which of them have a community of cause so that they might yield to a single measure for improving the dietary? Can we usefully delimit our problem so as to narrow its scope?

Scurvy in its heyday was a disease of sailing ships, polar explorations and beleaguered armies. It is not a disease of prime importance for the populations of the underdeveloped portions of the world. Where it occurs among such populations it must be dealt with by special measures, notably the increased use of native fruits. We can set scurvy to one side.

Rickets likewise is properly and strictly speaking not a dietary disease. It arises primarily from a lack of sunshine on the human skin. Our bodies have the capacity to deal with it if they have access to sunlight. The calcium phosphorus ratio of the dietary of course affects the occurrence of rickets but, in view of the predominant role of sunlight, I think we may put rickets beside scurvy as something for special consideration where necessary.

Xerophthalmia and other manifestations of vitamin A deficiency also require special consideration. In the West liberalized supplies of butter or fortified margarine is the best remedy for vitamin A deficiency. In Asia and in most of the underpriveleged world this measure won't work, for mostly the people do not use bread; and if they do, they do not spread anything on it. In Asia where vitamin A deficiency is not uncommon and often is severe, resort must now be had to green and leafy vegetables of native origin. If the deficiency is to be supplied through fat, it must be through cooking fat. Fortification of cooking fat requires protective antioxidants, not yet fully developed by Western technology. Synthetic vitamin A is cheap enough to compete readily with any food source, though Asian countries are short of foreign currencies which may be required. All in all I think we are justified in putting xerophthalmia to one side along with rickets and scurvy.

Our analysis leaves beriberi, pellagra and ariboflavinosis as 3 diseases which might yield to some common measure having to do with the use of cereal grains. Beriberi and pellagra are both due to the too exclusive use of certain grains in the dietary. In beriberi, white rice or white flour is the cause; in pellagra, white flour, maize or cassava. Ariboflavinosis cannot be said to be caused by the wrong use of grain. In the West the chief available remedy for ariboflavinosis is abundant supplies of milk, a product which in Asia is destined to be in very short supply for decades or perhaps centuries to come. One can search the food composition tables in vain for a likely source of all the riboflavin which the Orient seems to need. It is doubtful whether all the present vegetable and cereal crops of the world produce an adequate supply of riboflavin for the purpose. One has to turn to animal food products to find it, and feeding Asia to any great

extent on animal food products is clearly impossible. Either we shall have to revise radically downward our present estimates of the human riboflavin requirement or else conclude that the only way to meet the world's needs for riboflavin today is by synthesis. This would indeed be a most startling conclusion, to wit, that the human race has evolved through the long centuries without any means, until October 1943, of completely fulfilling one of the basic requirements of the entire race. The proposition does indeed require serious study.

The measures which are available for the prevention of beriberi, pellagra or ariboflavinosis are:

1) general improvement of the dietary by inclusion of a greater variety of foodstuffs carefully chosen to give balance; *2*) the use of undermilled rice; *3*) the use of parboiled rice; *4*) the use of methods of cooking foods to preserve the content of the vitamins; *5*) the enrichment of rice or maize with appropriate vitamins; *6*) the use of synthetic vitamins in other ways.

Note: Only methods 1, 5 and 6 are applicable to all 3 deficiency diseases. Methods 2, 3 and 4 offer little promise against ariboflavinosis and none of them have much bearing on the occurrence of pellagra. They are methods adapted to reduce beriberi only.

The 3 deficiency diseases we are emphasizing do not of course occur prevailingly together. Beriberi and ariboflavinosis affecting the rice-eating populations of Asia and elsewhere offer some menace wherever white wheat flour is a staple. Pellagra is less widely prevalent and threatens principally those peoples who live largely on maize as in parts of Yugoslavia, Roumania and Egypt. It is notable that the Indian populations of Latin America have a high predominance of maize in their dietaries yet there is little pellagra among them. The meager incidence of pellagra in Mexico, Guatemala and the mountain regions of South America must be ascribed to 2 things: *a*) the combination of beans with maize which is so generally popular and *b*) the addiction of the population to highly roasted coffee which can supply ample niacin to reinforce the diet in which maize so greatly predominates.

It is also notable that the maize-eating populations of Asia are not very subject to pellagra. Maize is eaten jointly with other cereals very widely in India, China, Indonesia, especially the Celebes, the Philippines, notably in Cebu and parts of Mindanao yet pellagra is rarely, if ever, reported. Where maize is combined in more or less equal proportions with rice or other cereals, pellagra rarely appears.

The problem of eradicating beriberi, pellagra and ariboflavinosis shakes down primarily to a problem of eradicating beriberi in very large populations and simultaneously attacking pellagra in quite restricted areas. We need not do anything about pellagra in Mexico, Guatemala and in the Andean areas of South America. Indeed, it would be impossible to do anything immediately effective about it for the maize is all raised in little family plots and the grain is milled by hand in the home. The only possible corrective measure in those areas is the encouragement of the use of a more varied dietary, the results of which will inevitably be exceedingly slow both there and in all underprivileged areas.

In Yugoslavia, Roumania and Egypt the deficiency of niacin can be readily attacked by adding niacin to the corn meal at the mills. This has already been done successfully in one area in Yugoslavia (1) and the only bar to its general application in that country is the indifference of the central government to its responsibility to furnish a domestic source of niacin.

Beyond doubt in the area of deficiency diseases in Asia, the greatest single need is the eradication of beriberi. For this purpose only 3 expedients can be considered possible for early adoption; namely the use of undermilled rice, parboiled rice or enriched rice. Of these 3 I am convinced that enriched rice is the simplest, cheapest and quickest to apply. The use of undermilled rice is well adapted to Japan where the mills are all small and scattered through every village and neighborhood because of universal electrification of all areas. There is also well established in Japan a very systematic and skillful fumigation of all rice warehouses and mills against the attacks of weevils. Fumigation is much more advanced in Japan than it is in the wheat milling industry of the United States. Fumigation is almost unknown in Asia outside Japan, Korea and Formosa.

Undermilling is also fairly successful in Indonesia, where the rice milling industry remains primitive and a very large part, about 80%, of the rice is still milled by hand as a domestic operation. Such rice mills as operate are all small and each provides rice for only its immediate neighborhood. Time in transit from mill to kitchen is thus rendered very short so there is little opportunity for weevils to propagate in the rice after it is milled. The conditions which prevail in Indonesia, because of its very meager industrial development, also apply to the hilly regions of Burma, Thailand, Vietnam and many parts of India where hand pounding of rice still prevails. These conditions are emphatically absent in the great, intensive rice-raising regions of Asia of which the most notable are those around the great rice ports of Asia: Rangoon, Bangkok and Saigon. In these big rice milling areas undermilled rice is unknown as a commercial product. If the big rice mills of those areas were to try to market undermilled rice overseas under present conditions, they would be ruined in a single season by the depredations of weevils. Wherever brown rice or undermilled rice has been a month in transit the numbers of weevils will rival the numbers of rice grains in the product on delivery to the consumer. Only the most thorough and skillful application of fumigants can possibly prevent this, and one cannot be sure that this will be successful even with hearty cooperation of the millers. Fumigation has been successful in Japan, Korea and Formosa but the warmer, wetter climates and the much longer time in transit from mill to consumer introduce much more difficult problems in the south.

If undermilled rice is ever to protect Asia from beriberi the place to begin is by introducing fumigation into the warehouses and rice mills of Rangoon, Bangkok and Saigon. If this were successfully done all the great cities of Asia would be protected from beriberi, for all urban populations get a substantial share of their rice from one of those 3 ports. All the rice shipped from these ports is now very white and constitutes the great perpetuator of beriberi. Moreover as industrialization and urbanization proceed in the Orient, all the population is slowly drifting toward the worst type of rice supply. Hand pounding simply cannot be preserved where factories exist offering employment to women. The advent of automobiles has nowhere been deterred by the fact that the purchase of gasoline requires cash while one can raise feed for horses on his own land. The efficiency of the gas engine determines the trend. All Asia will presently become urban and industrial and the mechanical milling of rice is certain to supersede hand pounding in one area after another, creating new crises of beriberi epidemics.

Parboiling as practiced in India is a very meritorious process which should continue to be preserved wherever it is successfully practiced. However, its extension for the protection of all Asia is hopeless. It is a practice well adapted to the long dry seasons of India. The parboiling mills simply shut down during the monsoons. Elsewhere in Southeastern Asia where rain may fall any day in the year, even during the drier seasons, parboiling requires fuel drying instead of sun drying. This produces a serious problem, for parboiled rice has to be dried very slowly and carefully. Sunshine is almost ideal for the purpose. Fuel drying therefore poses a chemical engineering problem of formidable proportions, and in most places the cost of fuel adds from 50¢ to $1.00 to the cost of the finished rice. This poor adaption of parboiling to most climates plus the distaste of many Asians for the flavor of parboiled rice severely limits its adoption. The process has been known and used in India for centuries but has never been successfully operated in other lands, except for the use of migrant colonies of Indians who acquired a taste for the product in their homeland. The one exception to this statement is the use of so-called converted rice in the United States. The story behind this success in America is very well known but complicated, and I shall not stop to comment on it except to say that the factors operating in favor of it in the United States do not apply in Asia. In short, parboiling is fine where it works but it is not going to extend itself.

If we go back to review the 6 possible means for eradicating deficiency diseases in Asia, I think we find ourselves reduced to the use of synthetic vitamins as the most promising attack on beriberi. Cereal enrichment is the best bet for beriberi; it works equally well for pellagra and is the only possible solution in sight for ariboflavinosis, however serious or negligible that disease may be. It is high time, in my opinion, that the U. N. agencies resolve their misgivings about the artificiality of that approach and agree to unite on cereal enrichment as the one most promising remedy for the plagues of beriberi and pellagra, the causes of which have been known for 22 to 50 years.

That enrichment will work has been amply shown by the studies of beriberi by Salcedo *et al.* (4) and of pellagra by Darby and associates (1). The failures in both cases have been due to inability to convince the highest political authority that the problem must be dealt with successfully. Vigorous support of our efforts by the U. N. agencies has been just what was needed to assure success on a national basis in each case. Instead of that we have had a fictional attempt to rewrite the facts about beriberi in the Philippines. The statistics of deaths from beriberi in the Philippines were rejected by the team of FAO and WHO experts sent to the islands in 1952 at the invitation and expense of the Williams-Waterman Fund (3). The inauguration of the collection of these statistics took place during the first decade of this century on the orders of and under the supervision of Victor Heiser, the most successful leader in tropical health in the world during that period. The statistics have been faithfully kept ever since, except during the Japanese occupation of the Philippines, and constitute the most complete and detailed record of deaths from any disease in all Asia. They are infinitely better statistics than any other country of Asia, except perhaps Japan, can produce about any public health problem, yet they were thrown out the window by the team of FAO-WHO experts for no assigned reason. (It seems that the team chose to disbelieve the figures. Fortunately the report of this team has largely been ignored in the Philippines and it has done little harm to the program there.) How much a favorable report and subsequent vigorous support by FAO and WHO might have done to bring about the success of rice enrichment on a national basis is of course a matter on which we can only speculate. The whole history of rice enrichment in the Philippines, however, shows that what has been lacking is endorsement and support at the top level of government. FAO and WHO could persuade governments far better and more effectively than any private agency.

As I see the matter today, with the report of the FAO–WHO team before me, a mistake was made by the team in trying to prejudge rice enrichment as a future measure for all Asian countries and in failing to recognize that success (or failure) on a national basis in the Philippines was in the balance. The team's greatest responsibility was to see that the venture in the Philippines should be carried to a final conclusion. If rice enrichment fails ultimately in the Philippines it is less likely to be tried elsewhere. If it succeeds in the Philippines the experience there will provide far better answers to the questions which the team could foresee in 1952. The team was obligated to make an objective appraisal of its actual or potential value so that the world would get the benefit of a thorough-going trial of the measure. Instead of that, the team damned the enterprise by re-

citing in detail all its misgivings, many of which are in my opinion imaginary. To convince you of the justice of this criticism, I quote in their entirety the concluding paragraphs of the report which appear under the title 'General Comment and Conclusions.'

"Great credit is due to those responsible for introducing enriched rice into the Philippines and for conducting extensive trials of enriched rice under difficult conditions. These efforts represent a pioneer attempt to solve a serious nutritional problem by novel methods. In principle, the enrichment of rice with nutrients needed by a population must lead to improvement in nutrition. Rice enrichment has the important advantages that it can be introduced fairly rapidly, that it benefits the whole community, that it does not affect the flavor of rice and that the amount and kind of nutrients added can be controlled to some extent.

"The team fully recognized these advantages but, at the same time, noted serious obstacles to the successful introduction of rice enrichment by the present method into many countries where rice is a staple food. Unless these obstacles can be overcome, enrichment by present methods may be found, in practice, to be of less general value for the nutritional improvement of the diet in these countries than might theoretically be expected. The reasons for this conclusion which take into account various factors existing in the countries concerned are as follows: (1) the present dependence of rice-eating countries on external sources for synthetic vitamins and other necessary chemicals and the considerable expense involved in paying for these; (2) the difficulty of insuring that premix is added to rice in correct proportion in the mills; (3) the need for an elaborate system of control, the cost of which must be added to the cost of manufacture; (4) the need for appropriate standards and suitably equipped laboratories to secure legal convictions in the case of non-compliance with the regulations; (5) the slightly greater cost of enriched rice compared with that of ordinary rice, which affects its sale to the lower income groups; (6) the complications arising from the inclusion or otherwise of homepounded rice in rice enrichment programs; (7) the losses of added vitamins which may occur when enriched rice is cooked in excess water, subsequently discarded, according to current practice in some rice-eating countries; (8) the need for intergovernmental agreements and standards when enrichment programs are undertaken in countries not self-sufficient in rice and depending to a varying extent on imports; (9) the present lack of knowledge of loss of added nutrients during storage under tropical conditions of temperature and humidity.

"Obstacles to the successful introduction of rice enrichment must, however, be considered in relation to those encountered in popularizing other measures, such as the use of under-milled or parboiled rice.

"It is pointed out that further research and investigation may result in modifications and improvements of present methods of rice enrichment, through which many of these disadvantages may be overcome."

The difficulty of insuring correct proportions of premix was not inaccuracy but willful violation by millers who did not fear prosecution. The need for an elaborate system of control was purely imaginary. Just counting the premix grains in a standard measure of enriched rice was always sufficient. Appropriate standards were already fixed and one central laboratory could easily do all necessary analyses. There was no protest whatever about the increased cost of enriched rice to the consumer. There were no complications from home-pounded rice. It is simply excluded from the provisions of the law. Losses of vitamins when rice is cooked in excess water are serious in Burma but not in the Philippines, Malaya, China or Japan as rice is not so cooked in most of Asia. Present knowledge of losses of vitamins during storage is wholly adequate to insure they are not at all serious. In short, the obstacles cited against rice enrichment were quite contrary to actual experience with one exception, namely the cost of importing vitamins from abroad. This disadvantage was cited repeatedly against the project by one economist, Amando Dalisay, who could not be persuaded that better public health has economic value. One would not expect FAO or WHO experts to share his mistake. The one real obstacle to rice enrichment at the time of the team's visit, and ever since then, was that it was impossible to prosecute any miller successfully for violating the enrichment regulations. No one has successfully been prosecuted yet, not at all because of difficulty of collecting evidence against the millers, but because no prosecuting attorney was instructed to prosecute violations. The real trouble was a lack of conviction on the part of officials outside the health service that the law should be enforced. This highly significant fact the team failed to note at all.

As time has passed the basic conditions for the success of rice enrichment have become progressively more favorable. The price of thiamine has come down from $160 a kilo to $40. At a conservative estimate of 1 milligram requirement per person per day the cost of a year's supply of thiamine per person is less than 1.5 cents. At the most liberal estimate of 2 milligrams daily per person the annual cost is less than 3 cents. Daily lessons in the schools on the subject of nutrition cannot be provided at that cost. The cost of riboflavin has similarly declined and that of niacin has remained steady so that the daily requirement of 10 milligrams of niacin can be bought in the world market for an annual cost of 3 cents.

The patents on the manufacture of vitamins are rapidly expiring and their production is now competitive. I suggest that the U. N. agencies should begin negotiations for the construction and operation of plants to manufacture thiamine, riboflavin, niacin and, perhaps, vitamin A as well, in one or more of the soft currency countries of Asia so as to have an indigenous supply. Thiamine is already being manufactured in Japan in quantities exceeding 50 tons a year. With an established Asian supply at minimum prices the way would be open for a widespread adoption of rice enrichment and where needed of corn enrichment. Riboflavin can be added where needed.

While negotiations are in progress and plants are being built and gotten into operation, surveys should be conducted to determine what areas should receive such attention. Ramalingaswami has already suggested that rice enrichment should be given a trial in Thailand. A second and third area where it should be tried promptly are the two great rice exporting ports of Rangoon and Saigon. A fourth is Andhra State in India. In countries such as Burma and Taiwan where the practice is to cook

rice in excess water, special studies will be necessary to determine what counter measures can be applied with effectiveness.

Trials of rice enrichment in other Asian countries need not follow slavishly the techniques which have been developed and used in the Philippines. The Japanese have come forward with dibenzoyl thiamine, a physiologically effective form of thiamine which is almost insoluble in cold water. Perhaps methods of rice enrichment can be devised which will dispense with the need for coating the premix grains to prevent loss in washing of the rice. By its omission the cost can be substantially reduced.

While I consider rice enrichment the more feasible measure in most countries of Asia, I would happily support a trial of undermilling in any country of southeast Asia, if that method should be found more acceptable to the government or the rice trade of that area.

In my criticisms of the U. N. agencies I do not wish to ignore the fact that they have had other large responsibilities apart from the deficiency diseases. Even a fanatic about beriberi must recognize that the great campaigns in Asia for the eradication or control of malaria, for BCG vaccination against tuberculosis, for the treatment of yaws and trachoma are thoroughly admirable and worthy of every endeavor they have cost. These infectious diseases are mighty foes of public health, public welfare and economic prosperity. The possibility of their conquest within a few years is awaited by all thoughtful Asians with bated breath. The work of FAO in collecting data about the food resources and food deficiencies of many countries has also been highly useful in giving us a better grasp of the world's total food problem. In my view, however, FAO needs very much to be able to point to some major victories which would give its purposes and activities a vigorous new momentum. Beriberi presents a magnificent opportunity for FAO which it should seize for its own welfare without further delay.

I wish to point out that the deficiency diseases are extremely insidious and that they are sapping the vitality of Asians to an unknown degree. My conviction is that there are scores of millions, perhaps hundreds of millions, in Asia who are suffering from mild beriberi and have done so for years and they still don't know they have it. If you had talked to Filipino mothers as I have, who had lost 6 to 10 babies in succession in infancy, you would realize what I mean by saying that hopeless people do not respond to encouragement. As a drain on the vitality of many people who suppose they are well, the deficiency diseases are a tremendous handicap to the struggling millions of Asia.

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Some aspects of the nutrition and physique of Bantu communities

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THE SUBJECT OF NUTRITION and physique, and the factors modifying them, is a vast one; hence, it will be possible to touch on only a very few aspects as they relate to Bantu communities. In this short paper it is proposed to mention a few points bearing on the physique and the nutrition of these people, to provide examples of the extent to which both aspects may be modified by local practices, and then briefly to discuss needs for the future. It will be understood that the writer primarily has in mind the Bantu resident in the Union of South Africa.

CERTAIN FEATURES OF BANTU PHYSIQUE

The term 'physique' has been defined as—'The physical or bodily structure, organisation, and development; the characteristic appearance or physical powers (of an individual or race)—a definition, it will be observed, which includes more than the aspects of weight and height.

GROWTH

Birth Weight and Prematurity

In 1944, Heynes and Hersch (19) collected single pregnancy records of 2298 labours of Bantu from 2 urban hospitals; 105 were stated to be unequivocally premature. The mean birth weight of the 2,193 babies was 6 lb. 12.4 oz.; 67–70 % of babies weighed between 5¾–7¾ lb., and 17 % weighed under 5½ lb. In another series (500) in which all mothers had antenatal assistance, mean birth weight was 7 lb. 4 oz. Among Whites of the same period, in comparison, a local series of 942 babies (excluding multiple births) had a mean birth weight of 7 lb. 8 oz., 5 % weighing under 5½ lb. (30).

In rural and peri-urban areas, average birth weight varies from region to region, and appears to depend primarily on economic position. Thus, in one semi-rural Bantu township, where almost all adult males were regularly employed at a steel works, mean birth weight did not differ significantly from that of White babies born into homes of artisan workers in Johannesburg (unpublished observations).

Speaking generally, although the gap is narrowing, the birth weight of present day Bantu babies (rural and urban) is inferior to that of present day White babies; the Bantu figure approximates to that of babies born in England, Belgium and France at the turn of the century (39).

The much higher incidence of prematurity in the Bantu (and other non-white populations) may not be of significance in regard to their future well-being. It is well recognized in Central and Southern Africa that children, whose weight at birth by ordinary standards would cause them to be classed as premature, are often sturdy and active and can be reared without the special care that has to be lavished on White children of the same weight (14). In Johannesburg, Kahn *et al.* (24) found that the survival rate among 1,000 premature babies (almost exclusively under 4½ lb.) without the use of incubators, special feeding, etc., compared not unfavourably with that of overseas White premature infants.

Growth of Very Young Bantu Children

Jelliffe and Dean (23) have stated that "African children of poorer groups, living under both rural and town conditions, show the following quite abnormal growth, as judged by weight gain, in the early years of life: i) First 6 months of life: excellent growth with sufficient protein and calories supplied by a good flow of uncontaminated breast milk, while the child is protected from many infections by antibodies from the mother during pregnancy. ii) Second 6 months of life: fair growth, but breast milk no longer sufficient for child's needs of both protein and calories, and added foods (if any) usually mainly starchy carbohydrates, with little protein. iii) Second and third years of life: poor growth, or even no growth, or loss of weight for long periods, due to the low protein, mainly carbohydrate diets (starchy foods, sometimes small quantities of breast milk, a little added animal protein, such as

cow's milk, meat or fish), and to the numerous infections that occur (including diarrhoea, malaria, chest infection). During this stage, protein calorie malnutrition may occur. The 2 most common clinical forms are kwashiorkor and nutritional marasmus." Although written from Kampala, Uganda, statements similar to the above have emanated from the Congo (34), Rhodesia (15), and South Africa (42, 25). In contrast, among Bantu groups in a more favoured economic situation—as in the study of Kahn and Freedman (25) on children of Bantu nurses, sisters, and health visitors—growth data (both height and weight) during the period of the study (1–11 years) were found to tally with those of Iowa City standards of 'superior American children' (21).

Growth of Bantu School Children

Speaking generally, the height and weight pictures of Bantu children (rural and urban) almost invariably are inferior to those of contemporary White children, both local and overseas (36). Broadly, data combined from several studies suggest that current height of Bantu children is of the same order as children in the United States (18) at the turn of the century, and of British children cited in the Board of Education Study of Newman (37) in 1928. There are exceptions, of course; for example, in 1949, in an investigation of Welbourn (52) it was reported that the mean height of the Kampala Bantu children examined at 8 years was greater than that of local White children of the same age group, and incidentally, coming from very good homes. Bantu data for weight at various ages are slightly inferior to those of American and British children in the studies just cited. In comparison with poorer White children, for example those of Dundee and Glasgow (39) in 1920, mean weights of Bantu boys and girls at 13 years are superior. Although certain investigations suggest that increases in both height and weight in Bantu children have occurred within recent years, insufficient information is available to learn of the extent of such improvements.

Height and Weight at Maturity

The question of salient interest is, since Bantu children are both under height and weight, compared with White children, is growth merely delayed (not necessarily a deleterious happening), or does the slower growth prejudice both height and weight at maturity?

The following table indicates that the height of Bantu mine workers of 18–40 years (the group on which we have most information) is little inferior compared with corresponding Whites of the same age group.

Additionally, we have found smaller groups of rural Pedi and Tswana Bantu males, 20–40 years, to have mean heights of 65.5 and 66.25 in., respectively.

Regarding Bantu women, data are very inadequate. Small groups (100 or so) of Pedi and Tswana Bantu women, 20–45 years, were found to have mean heights of 60.5 and 61.5 in., respectively. The mean height of Dundee mothers in poor economic circumstances studied in 1920 was 60.94 in. (39). In the investigation of Kahn and Freedman (25) the mean height of the privileged group of Bantu mothers was 62.3 in., which is closely similar to that of Canadian women 20–40 years, namely, 62.7 in., reported by le Riche and Pett (32). Within the limitations of the studies cited, neither Bantu men nor women appear to be markedly inferior in height to White subjects.

TABLE 1. *Height of Male Population Groups at Maturity*

Observer	Population Group	No. Examined	Height, in.
Turner (46), 1910	South African Bantu		
	East Coast Bantu	1,337	66.5
	Transvaal Basuto	521	66.0
	Cape Province Bantu	680	66.25
Walker (48), 1954	East Coast Bantu	1,100	66.35
	Transvaal Basuto	650	66.25
	Cape Province Bantu	720	66.1
Carlson (4), 1942	United States Army recruits	800,000	67.5
Clements and Pickett (6), 1952	Scotsmen, National Service recruits	1,303	66.82

With both sexes and at all ages over 1 year, Bantu are inferior in weight in comparison to present day Whites. Moreover, in contrast to the latter, limited studies suggest that after 20 years weight does not increase significantly with age. In a recent series of Pedi males and females studied, of ages 20–80+ years, throughout the decades, males averaged approximately 127 ± 3 lb. and females 122 ± 2.5 lb. If the definition of leanness of McLester and Darby (33) be used, i.e. 15% underweight, then compared with Whites of similar height, the majority of Bantu children and Bantu men are 'lean'; it is very unusual to observe obesity in Bantu men. On the other hand, Bantu women appear to be 'lean' only after middle-age; obesity during the childbearing period is not uncommon, and is regarded by Bantu males as a desirable characteristic.

CERTAIN MILESTONES OF DEVELOPMENT OF BANTU

Psychomotor Development in Very Young Children

At Kampala, Uganda, Geber and Dean (14) reported that newborn Bantu children are at a more advanced state of development, judged by the criteria used, compared with normal White children. Much of the activity corresponds to an age of 4–8 weeks. In further studies on the psychomotor development of older Bantu children these workers found that up to the age of about 3 years development is usually in advance of White standards, the degree of advance being greatest in the youngest children. These findings are not unexpected, because observations on Bantu children in the first year of life have already shown that the accepted 'mile-stones' of development—raising the head, standing, walking, and so on—are passed at an earlier age than in White children. Unpublished ob-

servations of a similar character have been made on Bantu infants in Johannesburg.

Menarche

Kark (26) studied 1038 Bantu schoolgirls from 4 different rural areas in South Africa in relation to whether or not menstruation had commenced. In all groups investigated no girls had commenced menstruating before 13 years, when only 1 girl had started. At 15 years, 40.5% of the girls were having menses; at 16 the majority (80%) had started, while it was only at 19 years of age that all the girls were menstruating. Kark discussed certain possible factors influencing menarche age, namely, nutritional state, disease and climate; she considered physique and health to be important influencing factors, noting that in the groups studied the menarche occurred a year earlier in areas of improved compared with less satisfactory nutrition. The latter conclusion agrees with various data given by Mills (35) who noted, *inter alia*, that in Norway between 1868 and 1935 the mean age of onset declined from 16.1 to 14.5 years, a change undoubtedly related to improved standards of health and physique. In a recent unpublished study on 1,000 girls in a Johannesburg Bantu township, Oettle and Higginson obtained a mean figure of 14.8 years. A current figure for British girls is 13.5 years (54). To throw further light on the possibilities of using this criterion (time of onset of menses) as an index of nutritional state, Oettle has initiated large scale studies on Bantu schoolgirls in several regions.

PHYSIQUE AND PHYSICAL PERFORMANCE

Physical Prowess at Work

In tribal life, it is usual for females to do the harder work—cultivating, weeding, fetching of water and fuel, 'stamping' or grinding of cereals, and other household tasks. Men are concerned primarily with hut building, overseeing of the cattle, ploughing and sowing. Although Bantu males pursue a more active life than White males, the former are not characteristically hard workers. Nevertheless, under conditions where heavy manual work is required, as for example with Bantu mine workers, their capacity, even without training, appears to be in advance of that of similar White workers (Personal communication, Dr. C. J. Wyndham, Director, Applied Physiology Unit, Chamber of Mines).

Motor Fitness

Schoolchildren. Le Riche *et al.* (31) carried out studies using the Harvard Step Test (a test of physical performance) on Bantu boys at a local institution for young delinquents. They found 90% to be in the "good, excellent, or superior" classes in comparison with 30% noted in a corresponding study on American private school boys. We have carried out further similar studies on rural Bantu children, reaching the same conclusion. A correlation was found between Step Test performance and the height and weight pictures in different rural Bantu schools.

Bantu young men. Working with Bantu mine workers, Drs. Morrison and Strydom (personal communication) found that performances of mining recruits compared with White students entering the University of Illinois (8) were closely similar. The following mean scores for Bantu and Whites were obtained: 'Chinning,' 8.55 and 9.81; 'dipping,' 7.79 and 10.67; running the mile, 7.17 and 7.11 minutes; and for 'push-ups,' 15.62 and 16.49, respectively.

Older subjects. In an unpublished study, Dr. P. J. Kloppers and associated workers have investigated Harvard Step Test performance by Bantu and White prisoners (long-term) at Pretoria Gaol. Habitual activity in the racial groups differed little, yet the Bantu showed significant superiority in performance.

FACTORS INFLUENCING PHYSIQUE

Genetic Factors

The extent by which genetic factors influence growth and ultimate height has occupied much attention in many countries. In an early careful study on *Poverty, Nutrition and Growth* (39), published in 1926, the following quotation appears. "The persistence of racial characteristics has been interestingly shown in the report of the Medical Department of the U. S. Army in the World War (1921), where statistics relating to measurements of draft recruits in the U. S. Army showed racial differences as to height and body build clearly marked in the inhabitants of the country, the Scot-American being tallest of the 8 European races listed, with an average height of 67.93 in., while the Italian-American had an average height of 65.03 in. Racial variation is also shown in other measurements."

In Southern Africa there are very tall Bantu groups, like the Watussi of Belgian Congo. There are the very short statured Bushmen of the Kalahari Desert, a non-Bantu African group who were dwelling in the South before the general Bantu invasion in the 17th century. There are numerous tribal groups with intermediate statures, but the extent to which such are influenced by genetics has not been studied. Investigations like those on Japanese children dwelling in the United States (17), and on privileged South African Bantu groups, as in the study of Kahn and Freedman (25) suggest, however, that the genetic factor is not of overriding importance in the determination of rate of attainment of height.

Nutritional Factors

The food resources of rural Bantu communities depend on the agricultural potentiality of their land (terrain, soil, rainfall, temperature, altitude, etc.), the presence or absence of crop and animal parasites, as

well as on the providence and intelligence of the peoples themselves. In urban areas, the diet consumed (especially the type of foods) depends primarily on economic position. Obviously, it is very difficult to generalise, since the nutritional situation differs so much from region to region, and even within the same region. But very broadly, where cereals (maize, sorghum or 'kaffir corn,' millet) are the main source of calories, as in South Africa, the energy value of the diet is unlikely to be grossly deficient, except among the very young. Gross protein may not be seriously low, but the intake of animal protein certainly is low. Further north, where cassava, sweet potatoes, plantains, and manioc, are staple sources of calories, the nutritional position is inferior to that where cereal consumption predominates. The nutritional situations in Central and Southern Africa in regard to the young are well discussed in *Kwashiorkor in Africa* (3), and, in so far as South Africa is concerned, in the series of papers by Fox (12, 13) on 'Nutritional foundations of agriculture'. Regarding other features of the diet, the intake of fat is low almost invariably. The intake of certain mineral salts and vitamins is influenced to a large extent by the amount of green leaves (morogo, m'fino) and vegetables consumed. As already indicated, diet may differ markedly in near-by regions, e.g. among the Masai and Kikuyu (38) of Kenya, and among Shangaans in Mocambique and those dwelling on the Highveld beyond the ridge of the Drakensberg. In centres of population, some 'westernization' of diet and manner of life is occurring; certain of the changes involved are mentioned later. It should be added that low intakes of certain nutrients, e.g. calcium by the general population (50) and vitamin C by babies (1), do not appear to be accompanied by demonstrable stigmata.

Zymotic Factors: Parasitic Infestation

In southern Africa, there are regions where parasitic infestations—blood borne, helminthic and protozoal—are endemic. Understandably, their presence and severity have a strong influence on nutritional state and physique. Malaria and trypanosomiasis no longer are public health problems in the Union, but west in Mocambique, and northwards in the Rhodesias, Nyasaland, and Kenya, these and like diseases aggravate the ill-health picture when due to unsatisfactory nutrition. Bilharzia is rife in many regions, but apparently the ill effects are less marked in the Union compared with neighbouring territories. The same applies to ancylostomiasis (hookworm) and other helminthic infestations, which appear to be much greater problems in the coastal regions compared with the inland Highveld. Here again, the parasite situation can differ markedly in near-by regions. In studies on certain Bantu school children in Western Transvaal almost three quarters were found to be free from helminthic infestation. But 50 miles or so further west almost all children studied were parasitized, some heavily so. (Personal communication, Dr. J. J. Theron, National Nutrition Research Institute.)

EXAMPLES OF THE INFLUENCE OF LOCAL FACTORS AND PRACTICES ON NUTRITION AND PHYSIQUE

Babies

The following examples indicate how prejudicial local practices may be in relation to infant nutrition. In earlier days, according to Keen (27), 30% of Swazi babies died during their first year, the majority succumbing during the first month. There were many possible causes of this—malaria, syphilis, bad hygiene, ignorance, etc., but Keen stated that the main cause was the terrible custom among the Swazi that a new born baby was not put to the breast until the umbilical cord had fallen off. This takes about 5 days, and during that time the baby is fed on soft maize meal porridge, made into a very thin paste and just pushed into the baby's mouth. As a second example, many Zulu (22) mothers believe that their colostrum is not healthy for the baby, so they treat their breasts by squeezing them and applying hot fomentations for 2–3 days, or even as late as 5–6 days, before putting the baby to the breast. During this period weak maize paps are given, with or without sugar,—with understandable deleterious effects.

Children

The following study illustrates how influential a little known tribal practice may be on the growth of each sex of school children.

Over the course of a year, Keen (27) made observations on groups of Swazi school children (boys and girls) at boarding and day schools. The boarding school group grew as expected. However, although the day girls grew regularly, the day boys gained practically no weight during term time. The tremendous difference between the day boys and girls was investigated. On referring the facts to the principal, it was noted that the day girls brought food with them, yet very few of the day boys had anything to eat at midday. Ultimately, it transpired that the Swazi male is not allowed to carry food, this being the prerogative of the female. The Swazi boy would rather go hungry all day than degrade himself by carrying food to school. Here therefore was a little known Swazi custom which obviously accounted for the difference in growth rate between the boys and girls.

Girls and Pregnant and Lactating Women

There are numerous reports bearing on abstinence from valuable foods by girls, pregnant and lactating women, due to tribal customs. The young adolescent Zulu girl (22) is forbidden to consume eggs, since it is believed that females (especially young adults) become excessively fond of men as a result of consumption of this foodstuff. Eggs, moreover, are believed to lead to

barrenness. Among the Mashona of Southern Rhodesia, Jali (22) refers to certain tribes among whom mothers throughout their pregnancies go without milk, this foodstuff being taboo.

The Effects of Urbanisation

The effects of urbanisation are widespread in their ramifications. Certainly, medical treatment and hospitalisation facilities are far in advance of such in rural areas, and urban Bantu are becoming increasingly hospital conscious. On the other hand, tribal discipline is greatly weakened, and promiscuity and venereal disease, relatively rare in most country regions, increase markedly with urbanisation. Concerning nutrition, whole grain or high extraction cereals progressively give way to white bread and sugar, a pattern all too familiar to those acquainted with the impact of White civilisation on backward peoples (White and non-White). The whole subject is so enormous that the relevant ramifications on nutrition and physique can scarcely be touched upon in a short space. However, 2 examples of changes will be given. *1)* It is usual for Bantu mothers to produce breast milk adequate in amount and satisfactory in composition (47). In urban areas, however, the practice is being replaced by the use of proprietary products, prepared almost invariably in too dilute a manner (49). Even in Central Africa, Watt (51) states "Unfortunately dried milk preparations, widely advertised by press and hoardings, are gaining an unwelcome foothold." Unless prepared correctly, such preparations cannot but favour the development of infant malnutrition or even semistarvation. *2)* In rural areas, Bantu infants wear the minimum of clothing, and hence are exposed to abundant radiation. In such regions, many able clinicians of long standing have yet to see severe rickets. On the other hand, the crowded conditions in urban townships together with overclothing favour the occurrence of rickets, which is very common and often severe (10). This same situation prevails in similar centres of population elsewhere (50), e.g. in India, China, etc., and until recent decades, western cities such as Glasgow (11).

DISCUSSION

From the foregoing, several points seem apparent. Although the position differs somewhat from region to region, South African Bantu babies are lighter at birth, and grow slower than White children. Ultimate height for males and females is not markedly inferior to that of Whites. Weight, however, is lower in all groups, especially in males and females after middle age. In psychomotor development, new born Bantu babies are precocious, this feature extending into the 3rd year. In Harvard Step Test performance, studies indicate that the Bantu (children, young men, older men) are superior to Whites. Bantu girls are significantly later in starting their menses, although the gap between their onset and that of White girls is decreasing. In pregnancy, Bantu mothers appear to fare at least as well, and almost invariably they lactate better than White mothers. Yet, among these people intakes of nutrients, in terms of orthodox recommended allowances, are inadequate, sometimes grossly so; zymotic diseases, of varying intensities, exact their toll; moreover, numerous tribal practices can have a deleterious influence on nutritional state and physique.

What then of the future? What are we to seek to do? For the philosophy of proceeding slowly, various points have been considered from time to time.

Among Bantu young children,—the syndrome which causes perhaps most anxiety in relation to mortality and morbidity is kwashiorkor. Nevertheless, although long term studies are few, there appears to be no evidence that patients recovering are *ipso facto* seriously stigmatised in later life (43, 53). Furthermore, in this connection, it would also appear that slower growth in older children due to temporary adverse nutritional conditions (as with German (2) and French (44) children during the last 2 wars) does not necessarily prejudice future growth once the situation improves. Cathcart (5), quoting an earlier authority, once asked "Should we aim, as some enthusiasts would have us do, at feeding children in such a way as to produce the maximum growth and development of which each child is capable? If we succeed in this are we sure that we have benefited the child? Does maximum growth make for health and longevity? There is certainly some evidence that it does not. . . ."

Carlson (4) stated that of the first 800,000 U. S. A. selectees in 1941, the mean height was 67.5 in., the same as for the 1917–1918 draftees; but the 1941 recruits averaged 8 lb. heavier. During that intervening period some have feared that physique deteriorated (7, 40). The question arises, was that gain in weight essentially beneficial?

Keys (29) has pointed out "at the same height and age, Britons tend to be lighter than Americans, and the age increment in weight is smaller. In Japan, not only is the relative weight smaller, but the age increment in weight is much smaller than our own. A continuous rise in body weight with age, such as is the situation in the United States, is not inevitable. Among relatively primitive people on islands in the China Sea, there is no increase of average body weight after twenty-five".

These points suggest that the provision of additional calories to the South African Bantu population from school age onwards may not be wholly rewarding; they counsel a policy of cautious change. Also to be borne in mind is the fact that the Bantu pattern of diet and manner of life are associated, *inter alia*, with a relatively low mortality rate from cancer (20), and an extremely low mortality rate from coronary heart disease (50). If possible, our well meant desires to help must not simultaneously promote increases in these and other diseases which together exact such a fearful toll of lives among western populations.

But as against this detached, conservative, and in some ways inhuman approach, it must be faced that in comparisons of vital statistics (infantile mortality rate, tuberculosis mortality rate, expectation of life, etc.) the Bantu are considerably inferior to Whites. In a paper of this type, however, it would not be appropriate to seek to elucidate and apportion blame to the various factors, nutritional and non-nutritional, responsible for the adverse aspects of the Bantu health picture, a picture, moreover, which in many respects is common to the majority of the world's population today. There is only room to bring to the fore the salient point which the writer desires to emphasize, namely, that with increased spending power and other relevant alleviating measures, it is imperative to increase the education of these people in the widest sense. It is not so much literacy that is at issue, since the great majority of these people already are literate; it is the aspects of education which will inculcate understanding, self-reliance, and responsibility. What is in mind will not be exemplified.

An increase in spending power will afford amelioration only if a portion of the increase in money is spent wisely. Thus, additional food may not be entirely beneficial if it consists wholly of white bread, sugar, soft drinks, sweets, etc., such being the pattern of change now being observed in centres of population in the Union. The obtaining of dried milk (whether purchased at retail price, reduced cost, or gratuitous) will not be of maximum value in the post weaning period of Bantu infants if mothers almost invariably prepare the foodstuff for consumption in too dilute a manner, as is the case at present. In this connection it will be remembered how often it has been reported that in many regions where kwashiorkor is common the cause does not stem primarily from poverty, but from ignorance and apathy. The Bantu child after weaning, in contrast to the White child, has no priority at meal time in the family circle. Numerous other examples of this type could be given. In brief, as Keen (28) has stated, "without an intimate knowledge of Bantu psychology and tribal customs, our efforts in the nutritional field are doomed to failure. ... As far as the rural Bantu is concerned, the difficulties will not be overcome by animal experiments, laboratory work, or dietetic calculations, but only by a vast social and educational revolution." But it is not only in regard to nutritional matters that education is important. In the field of medical treatment, for example, Keen (28) relates the case of 1 of his patients who was 'bewitched.' Despite batteries of diagnostic tests and observations, nothing unequivocally wrong could be found. Ultimately, the young man went to a celebrated witch doctor in Natal, by whom he became 'de-witched', and then quickly gained health and strength. Although these occurrences are becoming less common, at least in urban areas, their elimination will not be accomplished quickly. Even in the field of medical research, one rapidly comes up against tribal inhibitions and taboos. One manifestation is reluctance to give samples of blood and stools (although not breast milk) for analysis. We have often had to give half a dollar for a little blood from non-hospitalised people from whom we particularly wanted samples. But most dramatic of all appears to be the reluctance to part with excreta, for fear of how its fate may affect the person's future. At this Institute, there are over 200 Bantu helpers. At one time the writer was seeking to carry out studies on the faecal lipids of these people. Yet even with incentives of half a dollar per stool and half a dollar reward to the 'boss boy' in charge, only 10 stools were obtained.

It would seem inescapable that all our endeavours in relation to practical improvements in physique and nutrition, in relation to reduction of zymotic disease (which Platt (41) regards as the more important task) and in kindred health matters, will be stultified, unless equally intense endeavours be made to educate the Bantu in the respects described. What is being emphasized, obviously, is far from new. It has been the burden of recommendations in numerous conferences and symposia bearing on the whole subject.

It will be understood, of course, that irrespective of changes in spending power, in health education, etc., several activities by both State and community are pressing on simultaneously, which when taken together and given time cannot but contribute to improvements in health. Thus, there are control measures against malaria, bilharzia, etc., and the provision of inoculations against smallpox, diphtheria, and polio; there is improved housing in urban areas, increased hospital services (especially in regard to tuberculosis) and antenatal and child welfare clinics; there is the subsidisation of the price of cereal foodstuffs, and the provision of certain foods at low cost (e.g. dried milk); moreover, the question of enrichment of cereal products is under very active investigation (16, 36).

It must be reiterated, however, that the value of all these measures will be limited unless the education of these people in health matters be improved, and their providence stimulated.

SUMMARY

Information has been given on certain aspects bearing on the physique of the Bantu—birth weight (and prematurity), growth of children, height and weight at maturity; certain milestones of development (psychomotor development, onset of menses), and motor fitness (prowess at work, physical performance). This was followed by brief general information on the factors likely to influence this picture (genetic, nutritional, zymotic disease, local tribal practices).

While the physique picture of the Bantu does not give cause for serious concern, the vital statistics situation is far from satisfactory. It has been suggested that the aspect most deserving of attention is the educational aspect in its broadest sense, without which the best ameliorative efforts by State and community will fall far short of their potential effectiveness.

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Malabsorption syndrome

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EVEN THOUGH the human diet may be as complete and optimal as possible, if there are digestive disturbances the useful effect will be only a fraction of the intended effect.

This holds in particular for disturbed absorption of fats, proteins and carbohydrates, as the aim of our nutrition is to continue the process of life by supplying the necessary energy to the body. Without doing injustice to the essential importance of vitamins and minerals, the nutrients required in the first place are the proteins, carbohydrates and fats.

Moreover, good absorption of carbohydrates, proteins and fats is of particular importance, because disturbed absorption of these main constituents of our diet not only causes difficulties in caloric respect, but also is the direct cause of the development of fermentative and putrefactive diarrhoeas. Even the whole body may eventually be attacked, due to irritation of the intestine and absorption of toxic products.

We therefore feel justified to limit ourselves in this article to the discussion of disturbances of carbohydrate, protein and fat absorption. We do not start from the well-known clinical pictures of the malabsorption syndrome to reconstruct the pathological processes in the gastrointestinal tract, but we would like to deduce, against the background of the physiological-chemical processes, which disturbances in the fat, protein and carbohydrate digestion may give rise to the development of malabsorption.

NORMAL FAT ABSORPTION

The absorption of fat holds a separate place, as neither fat nor its constituents the fatty acids are water-soluble. Emulsification and saponification are therefore necessary to enable the passage of fat through the intestinal wall. In the first place lipase is required, an enzyme mainly secreted by the pancreas but also by the cells of the intestinal wall. The intestinal flora, also, has a not unimportant lipolytic activity, but this is not of importance for the absorption of alimentary fat, because the bacterial flora is not present in that part of the intestine where fat absorption takes place.

In the duodenum, with its weakly acid pH, monoglycerides and diglycerides are formed from fat, by lipolysis, as also free fatty acids. Monoglycerides and diglycerides possess a markedly emulgating activity and, with the bile that is added to the chyme in the duodenum, and which also exerts an emulgating activity, they bring the fat that has not yet been split into a fine emulsion; thus further action of lipase on it is facilitated. Now the free fatty acids, the mono- and diglycerides and the few remaining triglycerides are absorbed and transported further via the lymph vessels. A part of the free fatty acids, especially the low-molecular ones, is absorbed as bile acid complexes and transported portally.

Apart from the presence of lipase and bile, good funcfunction of the cells of the intestinal wall is necessary for the good course of this absorption process (scheme 1).

Attention should also be given to the capacity of the intestine to select the different fatty acids. The low-molecular fatty acids C_6, C_8 and C_{10} and partly also C_{12} are, following absorption, transported to the liver via the portal route (1, 2). As these fatty acids belong neither to the human depot fat nor to the human blood fat, they are either metabolized in the liver or perhaps also used as components for the synthesis of higher fatty acids.

In a certain sense the fatty acids C_6, C_8, C_{10} (partially also C_{12}) therefore constitute a stress on the liver (5). The main part of the high-molecular saturated and unsaturated fatty acids C_{14}, C_{16} and C_{18}, on the contrary, is, after absorption, directly added to the blood, namely with the lymph via the thoracic duct, and transported to the depots. C_{12} holds an intermediate position; after absorption part of it is transported via the lymphatic route, and another part via the portal route.

In a healthy person the fat absorption is almost quantitative, i.e. the absorption coefficient is more than 95%. An exception is the absorption of mutton tallow, for which an absorption coefficient of 90% is normal. The group of infants younger than one year also constitutes an exception if they are not given breast milk (fig. 1).

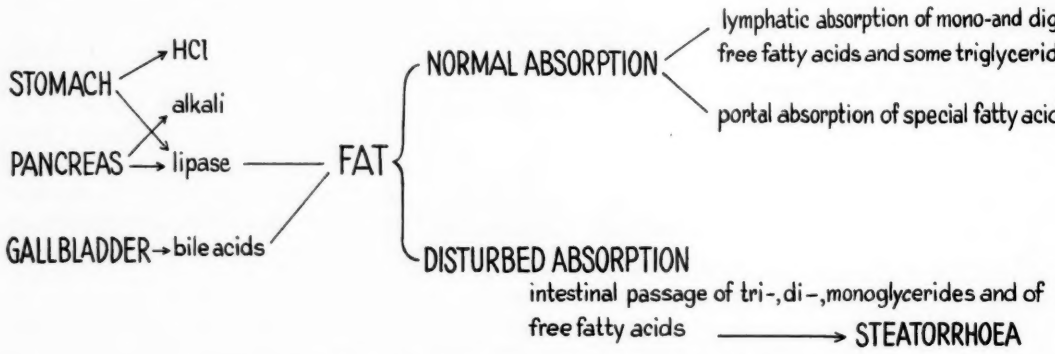
DISTURBED FAT ABSORPTION

Disturbed fat absorption, resulting in steatorrhoea, may therefore first and foremost be expected if there is a lipase deficiency, e.g. in cystic fibrosis of the pancreas, or if the lipase is insufficiently activated, e.g. in the case of biliary deficiency.

In this case the fat in the faeces should be expected to be unsplit; this occurs however only occasionally, as

FAT DIGESTION

(the digestion is disturbed by insufficient secretion or activity of one or more of the underlined substances or by impaired functioning of the intestinal cells)



SCHEME 1. Indicates possible errors in fat digestion causing a steatorrhoea.

the bacterial lipase usually still splits the non-absorbed fat, but too late, i.e. too far down the intestine to enable a proper absorption. It should be borne in mind that a normal splitting rate of the faecal fat of about 90%—i.e. many fatty acids in the faeces and few triglycerides—does not exclude cystic fibrosis of the pancreas by any means. If, however, many triglycerides and few fatty acids are excreted with the faeces, this is characteristic of insufficient lipase secretion by the pancreas.

The digestion of fat is not only influenced in an unfavourable way by the absence of lipase, but also by every disturbance in the bile secretion or bile composition (atresia of the biliary passages, gallstones, parenteral infection, etc.); this also leads to steatorrhoea.

Insufficient secretion of alkali by the pancreas also exerts an unfavourable influence on the absorption of fat, as the formation of biliary acid/fatty acid complexes requires an alkaline milieu.

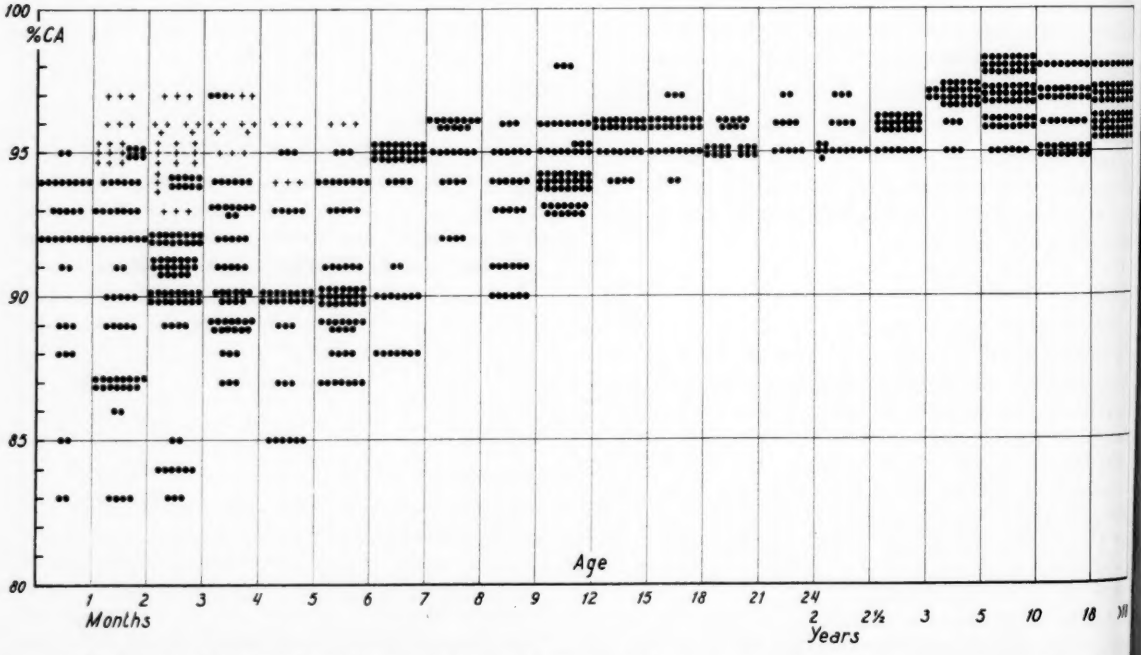


FIG. 1. Coefficients of fat absorption in *normal* babies, toddlers and children on cow-milk (• • •) and some babies on breast milk (+ + +).

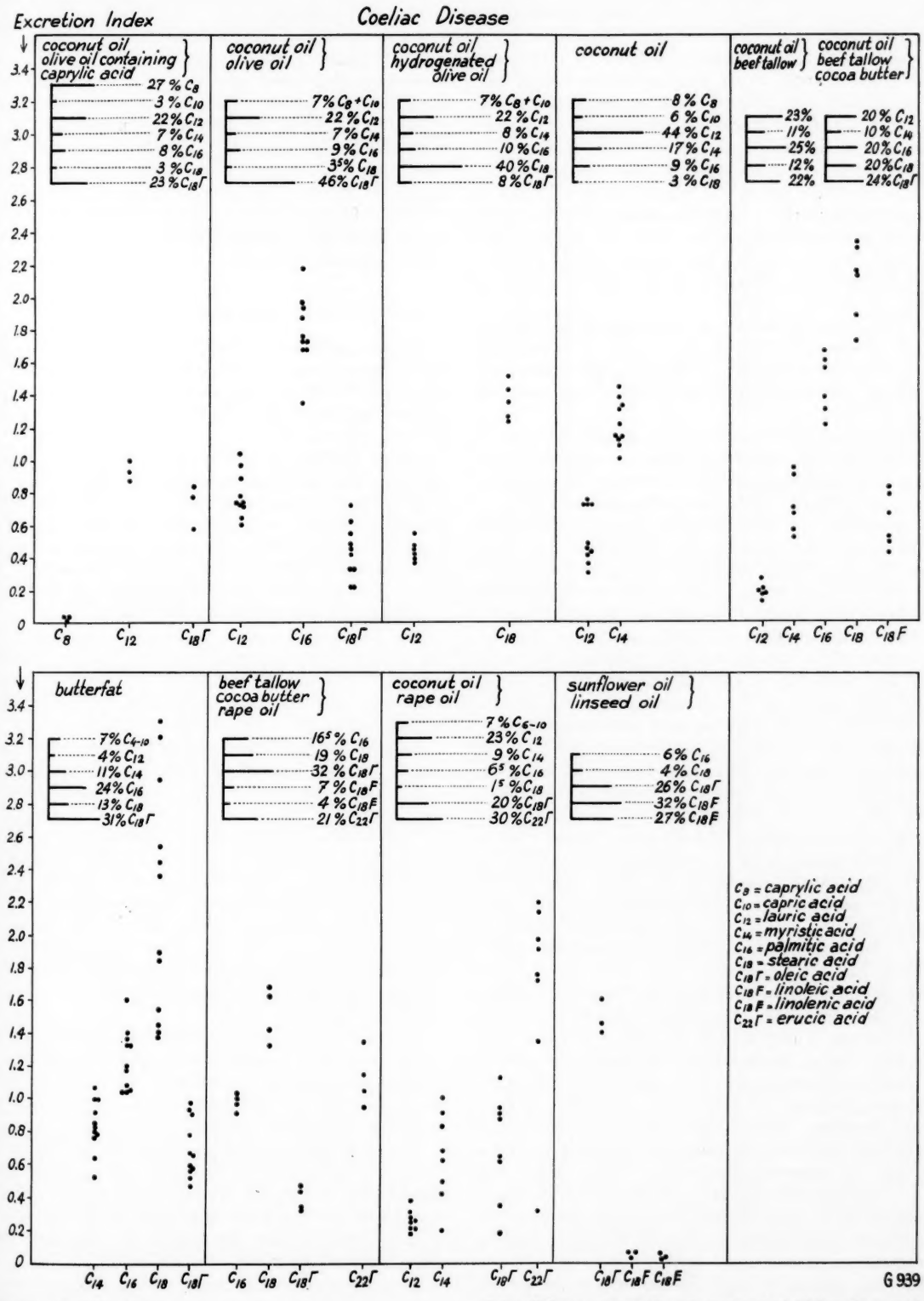


FIG. 2. Excretion Index

$$\left(\text{i.e. } \frac{\text{the coefficient of excretion of one particular fatty acid}}{\text{the coefficient of excretion of the total fatty acids}}\right)$$

of the different saturated and unsaturated higher fatty acids in patients suffering from celiac disease, consuming different fats. At the top of each figure the composition of the fat consumed is given.

Finally (but this is certainly not the least important factor), disturbances in the function of the cells of the intestinal wall may also give rise to steatorrhoea. In contrast to the steatorrhoea that develops as a result of a lipase or bile deficiency, this steatorrhoea is of a secondary nature. It arises, e.g., in celiac disease when a disturbance in the proteolytic activity of the cells of the intestinal wall causes a metabolic defect that results in an abnormal excretion of fat with the faeces. This secondary form of steatorrhoea is therefore considered in more detail in the discussion of the protein digestion below.

If, in the case of primary steatorrhoea, alimentary fat escapes absorption, it also takes with it fat-soluble vitamins, which means that, secondary, also a disturbed absorption of vitamins A, D and E should be borne in mind.

In every case of steatorrhoea one should further also be on the alert for a disturbed calcium absorption, as the higher fatty acids form an insoluble soap with calcium; thus much calcium can be excreted with the stools.

The excretion of fatty acids was studied in detail in celiac disease and cystic fibrosis of the pancreas (3). After determination of the different fatty acids excreted with the faeces, it could be calculated whether certain fatty acids were retained by the body by preference.

In celiac disease there is indeed a preference for the absorption of the unsaturated and low-molecular saturated fatty acids (fig. 2). Independent of the composition of the alimentary fat, the percentage excretion of the saturated fatty acids increases with increasing molecular weight of these acids. This means that relatively more stearic- (C_{18}) than palmitic- (C_{16}), more palmitic- (C_{16}) than myristic- (C_{14}), and more myristic (C_{14}) than lauric acid (C_{12}) is excreted with the faeces. For the excretion of the unsaturated fatty acids this sequence is as follows: erucic acid (1 × unsaturated C_{22}), oleic acid (1 × unsaturated C_{18}), linolic acid (2 × unsaturated C_{18}), linolenic acid (3 × unsaturated C_{18}). This suggests a selecting capacity of the cells of the intestinal wall or a specific excretion of fatty acids from the milieu intérieur into the intestine.

Moreover, it appears that in celiac disease there exists a certain competition in absorption between the different fatty acids (fig. 2). For example, oleic acid is excreted by a celiac disease patient in considerably smaller quantities if it is combined in the diet with the saturated fatty acids palmitic and stearic acid, than if the fat consumed contains, besides oleic acid, the unsaturated linolic acid and linolenic acid. This competition is for example also observed if lauric acid in the alimentary fat is combined with caprylic acid (C_8), or with higher saturated fatty acids or erucic acid. In the first case much more lauric acid is excreted in the faeces than in the second and third case.

In cystic fibrosis of the pancreas neither the preference for absorption of some of the fatty acids nor the competition phenomenon is by far as clear as in celiac disease (fig. 3). This is to be expected, as in this disease, due to a lipase deficiency, much undigested alimentary fat escapes absorption and is excreted with the faeces. Thus in cystic fibrosis of the pancreas the composition of the faecal fat resembles that of the alimentary fat.

In celiac disease this is definitely not so, as in this case, thanks to the good functioning of the pancreas, a good deal of the fat can be absorbed, the composition of the faecal fat therefore being largely determined by the results of selection and secretion of the fatty acids by the cells of the intestinal wall.

NORMAL PROTEIN ABSORPTION

Proteins must also undergo a hydrolytic splitting into their constituents, the amino acids, before they can be absorbed. While for the splitting of fats probably one enzyme suffices, several enzymes are required to split proteins (scheme 2).

While formerly a distinction was made between proteases and peptidases, enzymes that specifically split proteins and peptides respectively, at present the terms exopeptidases and endopeptidases are used. The exopeptidases hydrolyse only terminal peptide bonds, the endopeptidases split peptide bonds in the centre of the chain. The first show a great resemblance to the former peptidases, the latter to the former proteases.

The following exopeptidases are of importance for the human protein digestion: dipeptidases, leucyl aminopeptidase and carboxypeptidases. As regards the endopeptidases, here we are concerned with the action of pepsin, trypsin and chymotrypsins.

Some of the peptidases are regarded as metal complexes; Zn, Mg, Co and Mn have been demonstrated.

The gastric juice exerts only an endopeptidase activity by the action of pepsin.

The pancreas juice has both an endopeptidase and an exopeptidase activity (formerly together indicated as trypsin activity), endopeptidase activity by the trypsin proper and by chymotrypsin, exopeptidase activity by dipeptidases, carboxypeptidases and leucyl aminopeptidase (at the time usually called pancreas erepsin). Enterokinase, the substance that activates trypsin and carboxypeptidase, has to be present, since the enzymes are secreted as enzymogens.

The proteolytic activity of the cells of the intestinal wall is only based on exopeptidase activity, exerted by a number of enzymes also secreted by the pancreas: leucyl aminopeptidase and dipeptidases (as a mixture formerly indicated as intestinal erepsin).

The proteolytic enzymes produced by the cells of the intestinal wall do not act in the first place in the intestinal lumen, but in the intestinal cells themselves. The proteolytic activity in the intestinal lumen, as far as it is not caused by the pancreas juice, originates from desquamated cells of the intestinal wall.

The sequence of the amino acids, the presence of certain side-chains and the presence of certain terminal groups (—NH_2, —COOH) determine which of the enzymes mentioned just now is able to split the peptide

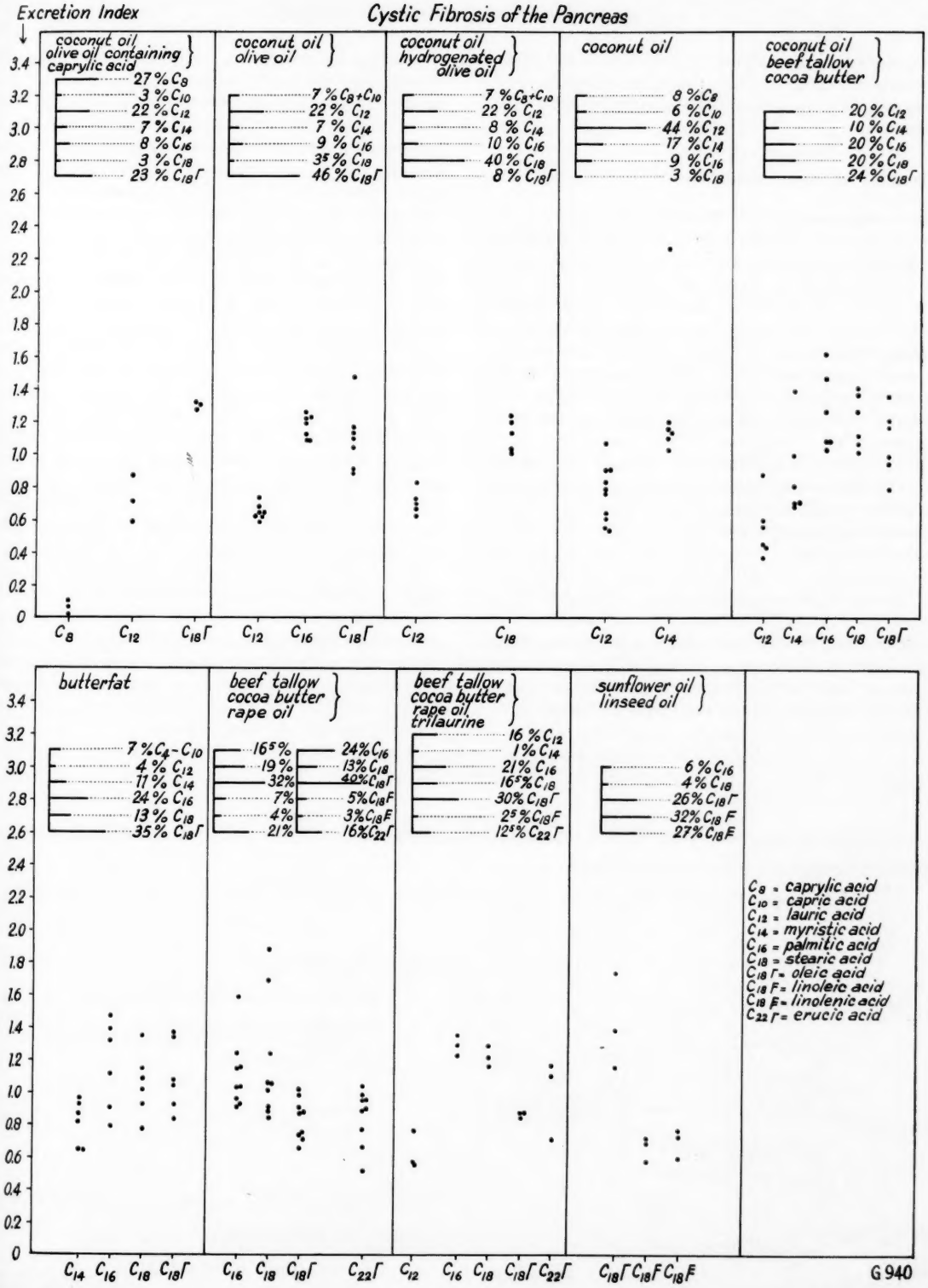


FIG. 3. Excretion Index

$$\left(\text{i.e. } \frac{\text{the coefficient of excretion of one particular fatty acid}}{\text{the coefficient of excretion of the total fatty acids}}\right)$$

of the different saturated and unsaturated higher fatty acids in patients suffering from cystic fibrosis of the pancreas consuming different fats. At the top of each figure the composition of the fat consumed is given.

bonds present in the various alimentary proteins. Finally by combined activity of the proteolytic gastrointestinal enzymes, the proteins are entirely broken down into amino acids.

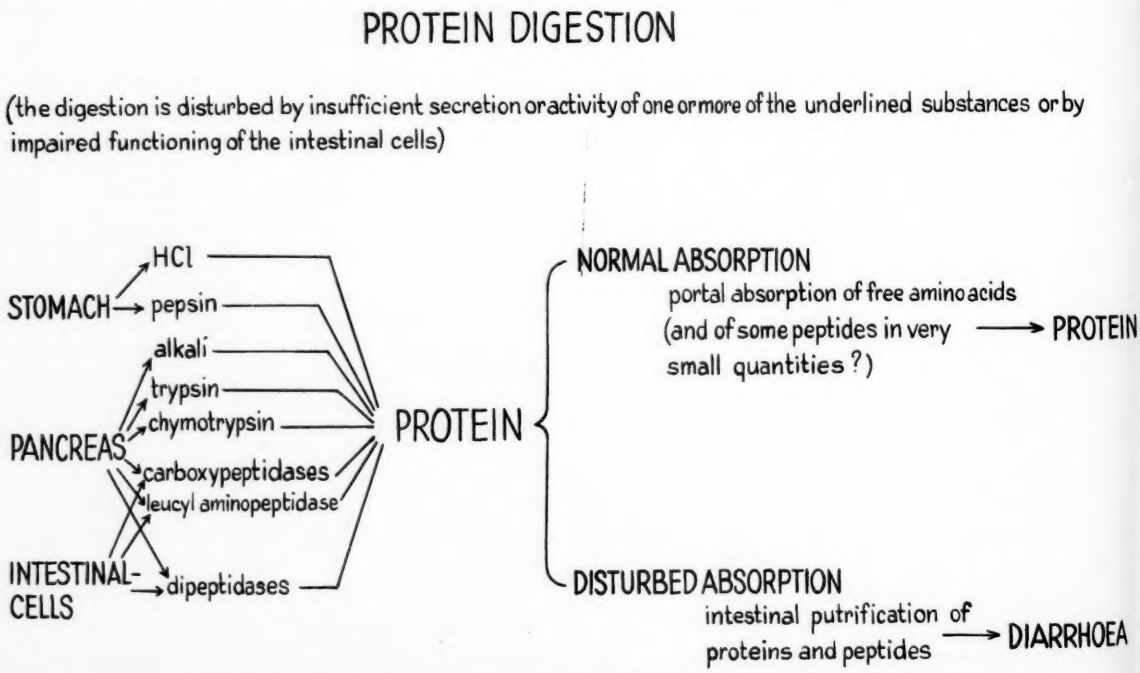
DISTURBED PROTEIN ABSORPTION

If, subsequently, the causes of disturbed protein digestion are studied, the first to be taken into consideration is the absence or diminished activity of the enzymes of the pancreas since a pepsin deficiency does not easily give rise to an important disturbance of the protein absorption, because the pancreatic enzymes are able to take over the activity of pepsin. Absence of enterokinase as an activator should also be kept in mind as a cause of disturbed protein absorption; as far as we know, however, up to now no such case has been reported in the literature.

As a result of a deficient proteolysis in the intestinal lumen, proteins and peptides fall victims to the intestinal flora. This results in a marked growth of the intestinal flora and in a stronger peristalsis, and the colon has, in an increasing degree, to elaborate the metabolic products of a putrefactive flora. The intestinal wall is irritated and formation of mucus takes place, which disturbs the reabsorption of water, combined or not combined with hypersecretion, finally resulting in a water-thin putrefactive diarrhoea. Moreover, toxic products get into the milieu intérieur, which leads to a general condition of intoxication that threatens the function of every cell of the body, including those of the intestinal walls. This not only exerts a bad influence on the digestion of the proteins, being already disturbed, but also on that of carbohydrates and fats, the absorption of these also becoming worse and worse. But the absorption of the water-soluble vitamins and minerals also suffers as the diarrhoea aggravates. In this way the malabsorption syndrome develops, in the case of insufficient supply of proteolytic pancreatic enzymes.

The protein absorption may however also be disturbed, whereas the function of the pancreas is very good. This is the case when the proteolytic function of the cells of the intestinal wall is insufficient. Then the difficulties do not originate in the intestinal lumen but in the intestinal wall. In this case low-molecular peptides are not split into amino acids and they impair the metabolic processes in the cells of the intestinal wall themselves or, if they are absorbed via the blood circulation, one can imagine that they disturb the metabolic processes somewhere else in the body. The consequences of all this are that also the other enzyme activities cooperating in the absorption decline, and in this case, too, a malabsorption picture develops.

This is seen in celiac disease and non-tropical sprue. In these diseases the enzyme functions in the intestinal lumen are normal, as wheat protein pre-digested with pepsin and trypsin does not lose its disastrous effect in celiac disease and sprue patients (7, 9, 10). However, because the harmful effect of wheat protein disappears entirely if during the pre-digestion in vitro an extract of



SCHEME 2. Indicates possible errors in protein-digestion causing a putrifactive diarrhoea.

pig intestinal mucosa is also allowed to act on it, it is highly probable that in patients with celiac disease and non-tropical sprue the proteolytic activity in the cells of the intestinal wall is disturbed.

Moreover, it was shown that in the blood of celiac disease patients after loading them with gliadine—the protein component of wheat that is very harmful—the quantity of glutamine in bound (peptide) form increases (11), whereas this is not observed in normal subjects. This is also an indication for the absence of peptide-splitting enzymes in patients with celiac disease and non-tropical sprue.

In principle, however, it is not the wheat proteins alone that can cause such difficulties as seen in celiac disease and non-tropical sprue, for activity of proteolytic enzymes is required for the splitting of every protein. This may perhaps afford an explanation of the fact why a number of patients suffering from non-tropical sprue does not react entirely favourably to a diet that is, indeed, devoid of gluten, but that contains an excess of other proteins.

As is known, in celiac disease and in non-tropical sprue not only the protein digestion is disturbed, but also that of fat, as the patients produce a steatorrhoea. It should well be realized that this steatorrhoea is caused in quite another way than that observed in diseases in which the pancreas function is insufficient, as for example in cystic fibrosis of the pancreas. For in the latter disease there is insufficient lipase to split the fat, but in celiac disease, non-tropical sprue, etc. sufficient lipase is present. As there is also sufficient bile, the cause of the steatorrhoea in the last-mentioned cases should be sought for in a disturbance of the function of the cells of the intestinal wall. Primarily, the protein digestion is disturbed in these cells, but secondarily an incapacity to absorb fat in the right way develops in addition, resulting in a steatorrhoea.

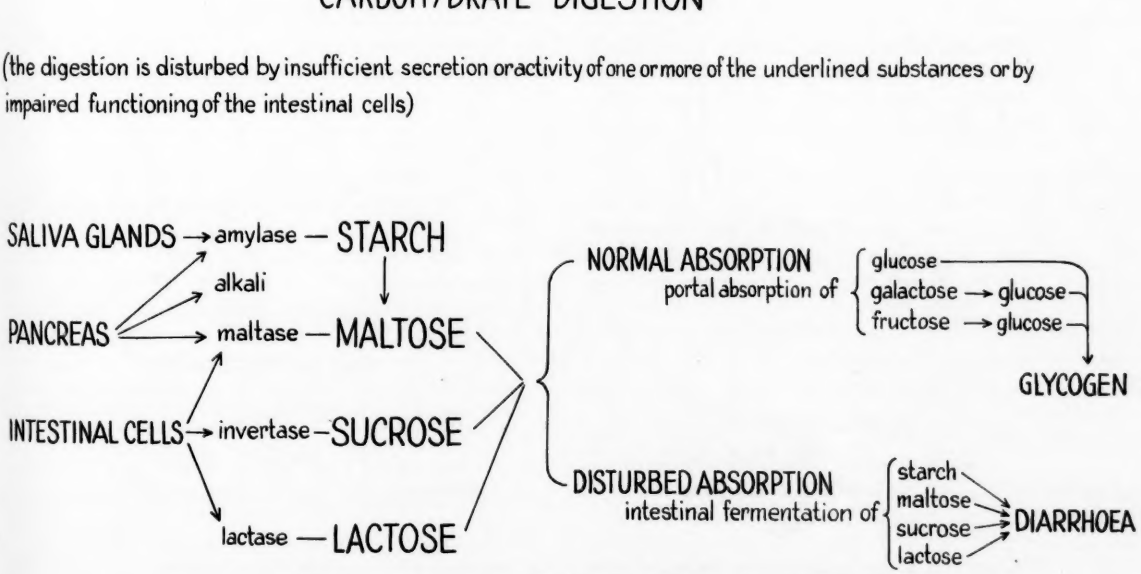
Therefore a celiac disease patient excretes a considerable quantity of fat in the stools when eating wheat, also if he is on a fat-free diet (11). This excreted fat cannot be alimentary fat. Here we are therefore dealing with an excretion of fat from the milieu intérieur, caused by a disturbance of the protein digestion in the cells of the intestinal wall. We know nothing of the mechanism of this process, which perhaps also exists in other diseases of the malabsorption syndrome.

It is interesting that some authors are of the opinion that probably also under normal conditions fat is excreted from the milieu intérieur into the intestinal lumen (8). This fat is reabsorbed via the lymphatics. In celiac disease and non-tropical sprue—apart from a disturbed absorption of the fat consumed with the diet—it seems probable that this reabsorption is disturbed by the impaired digestion of wheat protein; this being then also the cause of the fat excretion on a fat-free diet containing wheat.

NORMAL CARBOHYDRATE ABSORPTION

The number of carbohydrates generally used is limited to one polysaccharide, starch, and three disaccharides, maltose, sucrose and lactose.

These carbohydrates cannot be absorbed as such, but only as monosaccharides. One enzyme is sufficient to split all different kinds of starch that may be present in our nutrition, into maltose. Each disaccharide requires



SCHEME 3. Indicates possible errors in carbohydrates causing a fermentation diarrhoea.

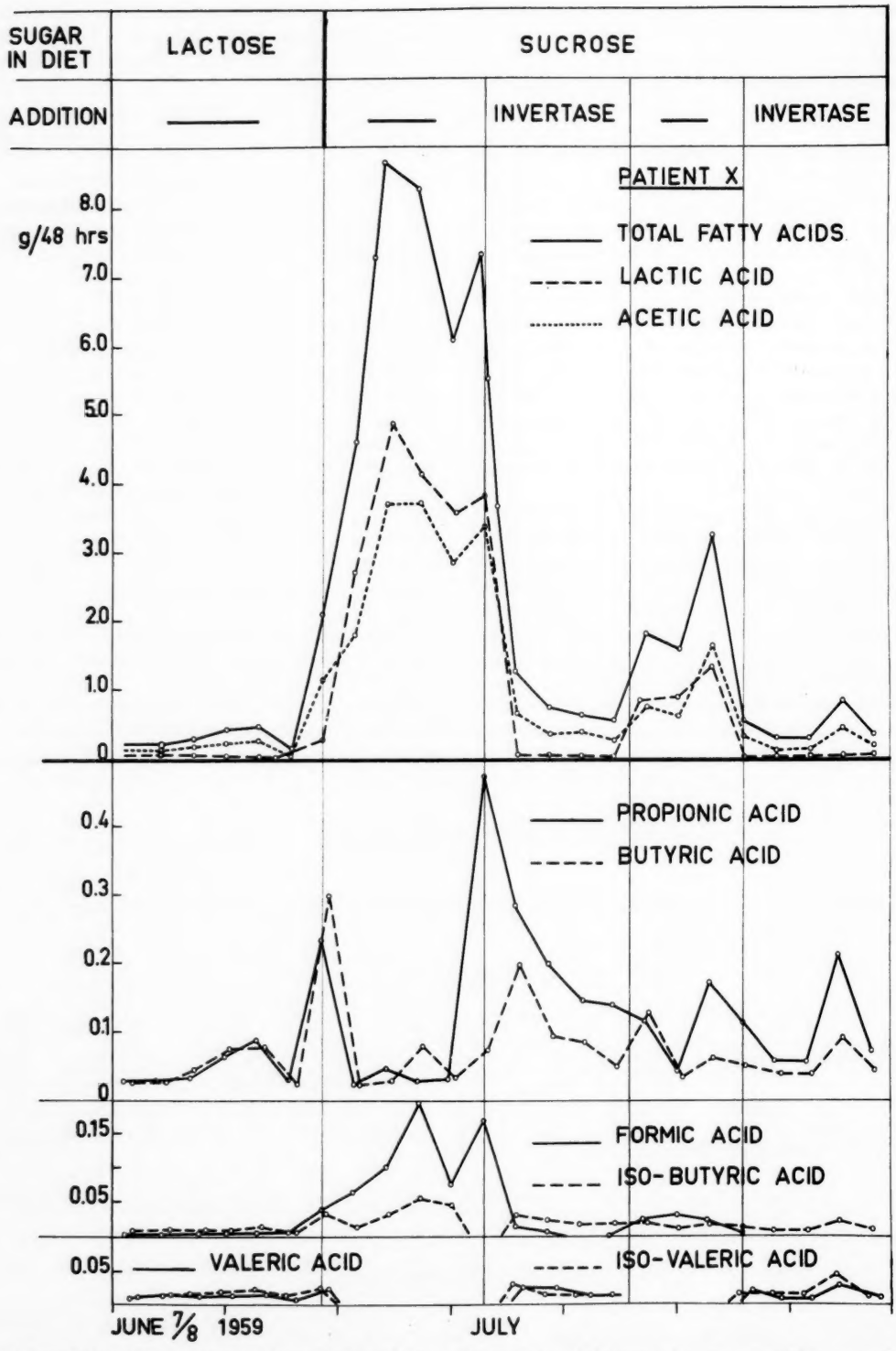


FIG. 4. Differentiation of the output of low-molecular fatty acids with the faeces of a child suffering from diarrhoea caused by invertase deficiency; the diet containing as carbohydrates lactose and sucrose (with and without addition of invertase).

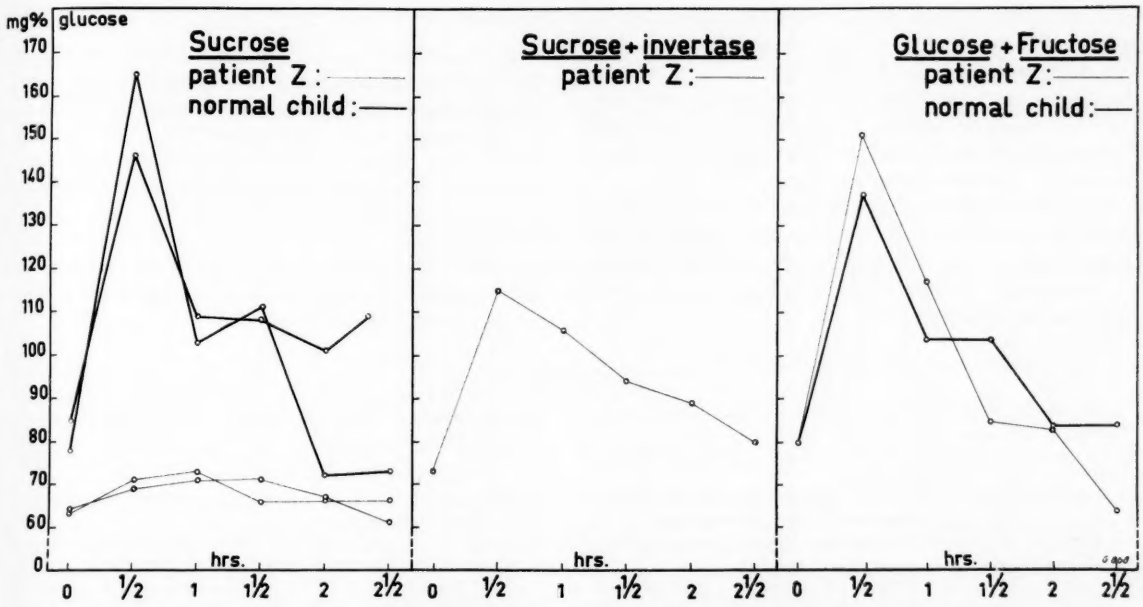


FIG. 5. Sucrose tolerance curves in a patient suffering from diarrhoea caused by invertase deficiency as compared with a normal child. *Left:* after loading with 2 g sucrose per kg bodyweight. *Middle:* after loading with 2 g sucrose per kg bodyweight, invertase added. *Right:* after loading with 2 g of glucose + fructose demonstrating that the absorption itself is not impaired.

however a specific enzyme, maltase, invertase and lactase, to cause, by hydrolysis, a splitting into monosaccharides, glucose, glucose + fructose, and glucose + galactose, respectively.

Some digestion of starch takes place already in the mouth and in the stomach under the influence of saliva amylase, but for the greater part starch digestion proceeds in the duodenum under the influence of the amylase secreted by the pancreas. The pancreas secretes, in addition, also maltase, but the cells of the intestinal wall do so to an even higher degree. Lactase and invertase are only produced by the intestinal wall cells (scheme 3).

DISTURBED CARBOHYDRATE ABSORPTION

If one or more of the 4 enzymes mentioned are absent, sugars pass the small intestine and are attacked by the bacterial flora further down the intestine. This results in fermentation. This is not only demonstrated by the faecal pH that decreases from 7–8 to 5–6, but also by the formation of lactic acid and low-molecular volatile fatty acids, which are excreted with the faeces as metabolites of the fermentative flora. These acids stimulate intestinal peristalsis, whereas also the fluid secretion is increased markedly, often resulting in a water-thin diarrhoea.

This type of malabsorption may be caused by the absence or deficient activity of *each* of the 4 carbohydrate-splitting ferments.

This is sufficiently known for amylase, in particular in dysfunction of the pancreas. The undigested starch is partially excreted with the stools, but for the greater part fermentated in the intestine.

But also a lack of lactase as a cause of malabsorption is known in daily practice. Two children are described who both suffered from diarrhoea if their diet contained lactose (6). The diarrhoea disappeared as soon as this carbohydrate was withdrawn. Further, after loading with lactose it was demonstrated that the reducing power of the blood did not increase, whereas in normal subjects lactose is split into galactose and glucose under the influence of lactase, these monosaccharides causing a rise of the blood sugar content.

Not long ago we observed that a chronic diarrhoea may also be caused by the absence of maltase and invertase (12).

The cases concerned were a few pre-school children who suffered from chronic diarrhoea and who did not react to the current therapeutic measures; not a single indication of the cause of the diarrhoea was found. As the diarrhoea observed in the patients was thin, foaming and bulky, and contained very finely distributed mucus (which is typical for a diarrhoea originating in the small intestine (4)), we had to give serious thought to an absorption disturbance as a causative factor. Especially in view of the foaming and the low pH of the faeces we thought of fermentation as a result of a disturbance in the carbohydrate absorption.

In one of the patients the diarrhoea was indeed based on a deficiency of maltase and invertase; the lactase and amylase activities were normal in this little girl. In the 2 other children the diarrhoea was caused by the ab-

sence of invertase only. Recently we have been able to confirm the observation already mentioned that a lactase deficiency may also be the sole cause of a chronic diarrhoea. Both the blood sugar tolerance curves and the determinations of lactic acid and of the other various fermentation acids proved the correctness of the diagnosis in the patients concerned (figs. 4 and 5).

In a clinical respect, excluding from the diet the sugars involved was sufficient for the complete disappearance of the diarrhoea, whereas the invertase deficiencies were also efficiently counteracted by adding an invertase preparation to the diet.

SUMMARY

There are may causes for the development of the malabsorption syndrome but in our opinion disturbances in the enzymatic digestion of fats, proteins and carbohydrates occupy a very important place. We have discussed how these enzyme deficiencies may cause malabsorption, against the background of the physiological-chemical processes in the small intestine. It seems probable that a lack of activity, of no matter which representative of the vast group of fat, protein and carbohydrate splitting enzymes, may provoke the clinical picture of malabsorption.

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Neuromuscular disease in relation to nutrition

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MUSCLE WASTING AND WEAKNESS as an early sign of undernutrition (either acute or chronic) has been recognised from the earliest times but it is only with the development of histopathology and biochemistry during the last century that knowledge of the mechanisms responsible has been accumulating. The damage may be in the muscle cell itself, at the myoneural junction, or the primary lesion may be in the neural tissue with only secondary effects on the muscle cells. The neuromuscular syndromes which may arise from malnutrition vary considerably with the factors responsible for the disordered nutrition. There may be total caloric deficiency, protein deficiency with or without caloric deficiency, specific vitamin deficiency, again selectively or in combination with the foregoing, and finally mineral deficiency may play its part. It is in fact rare to find pure deficiency syndromes and more often the clinical picture is the result of a complicated series of biochemical disturbances, and varies if it results from acute shortage, chronic low grade deficiency or intermittent undernutrition.

It is necessary however to attempt a classification no matter how artificial it may be with the clear understanding that overlap or interplay of one with the other may frequently occur. The following grouping is suggested:

I. Syndromes due to vitamins and/or protein deficiency and/or mineral deficiency:

1) Specific vitamin deficiencies—thiamine, niacin, pyridoxine, pantothenic acid, B_{12}, vitamin E, inositol
2) Protein deficiency
3) Mineral deficiency—potassium, magnesium, calcium, phosphorus

II. Syndromes occurring in patients with a poor or marginal dietary but with no specific deficiency, factor demonstrable:

a) pyramidal tract damage
b) posterior column lesions
c) selective lower motor neurone damage
d) retrobulbar neuropathy
e) eighth nerve deafness

III. Syndromes where a toxic factor is recognized but undernutrition may play a contributory part, e.g. lathyrism, triorthocresol poisoning ('ginger' or 'jake paralysis').

With the time at my disposal I shall not attempt to review all these categories but I shall select mainly those of which I have some first hand knowledge and experience.

CATEGORY I

Specific vitamin deficiencies. As beriberi was the earliest of the neuromuscular diseases to be recognised as due to a specific vitamin deficiency more is known of the clinical picture, histopathology and the biochemical lesion than any of the other syndromes. There is no need to give a standard account of this disorder but it may be useful to emphasize gaps in our knowledge and to highlight some interesting clinical points from my own experience with many hundreds of cases in Changi POW Camp, Singapore, during the last war.

In spite of the enormous amount we have learned about the biochemistry of cellular metabolism in recent years there is as yet no satisfactory explanation as to why the nervous system and particularly the peripheral nerves should be so susceptible to thiamine deficiency. Handler (22) in an able review of the possible mechanisms by which thiamine deficiency might result in beriberi sums up the situation as follows: "From current knowledge of intermediary metabolism we could not possibly predict that thiamine deficiency would result in beriberi, nicotinic acid deficiency in pellagra and riboflavin deficiency in ariboflavinosis. Moreover current literature reveals very few experimental attempts to elucidate these relationships." The biochemists it would seem have still much to find out about the specific relationships of the highly complex enzyme systems in different cells, particularly those of nervous tissue. The histopathology of the neural changes in both experimental thiamine deficiency in animals and beriberi in man have been extensively studied but few new observations have been made in recent years. The view of Swank (51) that in peripheral neuropathy the cell body in the posterior root ganglion is the primary site of the disordered metabolism and that changes there lead to degeneration at the distal end of the axon is worth emphasising. It is supported by two well established facts *1*) that the longest and largest axons are affected first and *2*) that the earliest signs of damage to a peripheral nerve in beriberi appear in the areas most peripheral from the trophic nerve cell both in the central and peripheral 'axons.' This is best demonstrated in the

posterior root ganglion cell with an axon (strictly a modified dendrite) running up the posterior column (14). This thesis can be applied to all forms of peripheral neuropathy whether due to deficient, toxic or infectious factors. Most authorities agree that the varying presentations of beriberi depend on the degree of thiamine lack—when deprivation is sudden and complete gross neurological disturbances appear and death occurs rapidly with little or no histopathological changes in the CNS; when the thiamine supply is grossly inadequate—the acute picture of Wernicke's encephalopathy presents and the outstanding pathological change is in the endothelium of the blood vessels leading to numerous pin-point haemorrhages particularly in the brain stem. Finally if the deficiency is marginal with fluctuations above and below adequacy the peripheral nerve lesions results. This may be symmetrical or selective in its presentation. These are exemplified by the presenting neurological features in 171 of 400 cases of beriberi observed by the author. The remaining 239 cases had cardiovascular changes only (see table 1).

Denny-Brown (10) emphasises the interesting contrast in the sites of the neurological lesions in syndromes resulting from thiamine and B_{12} deficiency in man as well as experimentally produced pyridoxine and pantothenic acid deficiency in animals. In thiamine deficiency the distal parts of the first sensory and the lower motor neurone are most severely damaged, the latter usually more so. In B_{12} deficiency the dorsal and lateral columns and to a lesser degree the posterior nerve roots are affected, while in pyridoxine and pantothenic acid deficiency in animals the dorsal root bears the brunt of the damage. Again, if gross deficiency of these vitamins is produced rapidly the dominant clinical and pathological features are cerebral confusion and fits with pyridoxine and confusion alone with B_{12}. From a biochemical standpoint however, we still do not know why there should be this high degree of selectivity in spite of a rapidly expanding knowledge of the biochemistry of neural tissue. Cord damage is well recognised in classical pellagra but it is still not clear how much of this is due to specific niacin deficiency or to additional deficient, or even toxic, factors.

TABLE 1. *Analysis of Neurological Findings*

Motor symptoms only	38	(22.2%)
Sensory symptoms only	52	(30.8%)
Sensory and motor symptoms	81	(47.0%)
	171	(100.0%)
Motor features (with and without sensory changes)		
Weakness of the legs alone	85	(71.4%)
Weakness of the legs and arms	10	(8.4%)
Winged scapulae	3	(2.5%)
Weakness of muscles supplied by ulnar nerve in one hand	2	(1.8%)
Unilateral foot drop	19	(15.9%)
	119	(100.0%)
Additional symptoms		
Aching, pain, stiffness, tightness, or cramps in the calves or associated muscles	46	
Associated with motor weakness	37	
Associated with sensory changes only	9	
Reflexes absent or markedly diminished		
Knee jerks and ankle jerks	59	
Ankle jerks only	24	
Knee jerks only	4	
Knee jerks, ankle jerks and arm reflexes	9	
Total	96	

There is evidence that vitamin E deficiency and inositol deficiency can produce rapid muscular dystrophy in certain animals, e.g. rabbits and rats (12, 34) but there is no evidence as yet that deficiency of either or both plays any part in the muscular dystrophies that occur in man. Nor is there evidence that these disorders result from a specific biochemical defect in muscle cell metabolism. A genetic defect is undoubtedly present in many but how this leads to muscle cell dysfunction and ultimate death remains a mystery.

Protein deficiency. There has been much recent work on the effect of protein depletion on muscle-cell metabolism although remarkably little was known of the state of muscle tissue in malnutrition or cachexia before World War II. Vincent and Radermecker (52) recently reviewed the published work on the histology of protein deficient muscle and reported a careful study of their own. Bablet and Nomet (2) described in the myocardium severe degeneration of the fibres and lysis and disappearance of tonofibrils and striations, hypertrophy of nuclei and vacuolisation of fibres in kwashiorkor. Vincent and Radermecker (52) found the earliest changes to be an increase in the number of nuclei in the neurolemma and an increase in the size of the endothelial nuclei of the capillaries where they cross muscle fibres with small collections of lymphocytes round these vessels. In more severe cases there is loss of striation, increase in dark nuclei arranged in rows and ultimately the sacroplasm disappears.

The fine intramuscular rami of the nerves appear distended with oedema, occasionally there is an increase in the Schwann cells and adjacent fibroblasts but they did not consider the muscle changes to be neural in origin. In Jamaica, Waterlow and Mendes (53) have shown by biopsy of the pectoral muscle of children with kwashiorkor before and after treatment (1–3 months interval) that the ratio of noncollagen nitrogen to deoxynucleic acid phosphorus increased by 45% while the increase in body weight was only 29%. This gives an indication of the rate and amount of muscle protein repletion and therefore of pre-existing depletion. This discrepancy between muscle-cell protein and weight gain may be explained on the basis that during recovery, the gain in tissue protein is partially masked by loss of water and of fat. Experimentally in rats they also showed that sacroplasmic proteins are reduced whereas collagen is relatively increased, i.e. the loss of protein occurs at the expense of the cellular fraction (54). Garrow (16, 17),

TABLE 2. *Radioactivity (Counts Per mg. Nitrogen) of Organs in Normal and Protein-Depleted Rats in Relation to That of Serum (Activity of Serum Taken as 100 in Each Animal)*

	Days After Inject.	Liver	Brain	Other Viscera	Muscle	Carcass	Skin
Normal	1	79	26	62	22	19	17
Depleted	1	81	27	79	21	21	13
Normal	3	69	31	61	25	27	26
Depleted	2	80	25	56	9	16	9
Normal	3	69	41	63	34	34	27
Depleted	3	73	29	57	17	19	11
Normal	5	61	35	56	44	53	40
Depleted	5	85	38	55	23	24	15
Normal	7	83	70	72	55	58	83
Depleted	7	100	63	75	47	49	22

Average for 7 normal and 8 depleted rats.

as a result of studies on Jamaican infants and protein-deficient dogs, using methionine labelled with S 35 advanced the hypothesis that in protein depletion the synthesis of protein is concentrated in the more essential organs at the expense of the less essential ones. As Garrow's measurements were made only on blood proteins, Waterlow (55) measured the amount of labelled methionine incorporated in the viscera on the one hand and the skin and carcass on the other in normal and depleted rats. His results are summarised in table 2.

These results give some support to Garrow's theory. If this is so, the loss from muscle is relatively much greater than from 'essential' organs such as liver and brain. This is also borne out by further studies on Jamaican children (46) where the 24-hour creatinine output was measured in malnourished infants at intervals after loss of oedema. After 2 months on milk diets the output more than doubled. This increase in creatinine excretion was relatively greater than the increase in body weight over the same period. If creatinine output is a measure of muscle mass the deficit in muscle mass at the time of admission to hospital was considerably greater than the deficit in body weight. In the same infants measurements were also made at intervals of limb circumference and skin-fold thickness. From these, estimates were calculated of muscle bulk and fat bulk. Over the period of observation both of these increased relatively more than body weight.

Dreyfus, Shapira and Kruh (11), using radioactive glycine C^{14} in rats, demonstrated that the half-life of sarcolemmal-soluble proteins was 15 days while the insoluble protein (actinomycin) of the contractile fibres exhibited a stable radioactivity for 40 days. They suggest that this is the reason why the radioactivity of muscle is elevated for much longer than that of the liver and also that the fibrillary proteins do not draw on the common pool of amino acids. On the other hand when protein deficiency exists they may be mobilised and redistributed to 'essential organs.' There is still much to be learned about the biochemical changes in muscle and at the myoneural junction in kwashiorkor.

Mineral deficiency. The electrolyte changes in malnourished or kwashiorkor skeletal muscles are also of considerable interest. Gomez (18) reviewed these disturbances and depletion of potassium is one of the most striking that has been demonstrated both indirectly (23) and directly (44). This ion is very important in muscle metabolism and analyses of biopsy material (15, 53) have produced valuable information. Smith and Waterlow (44) have shown that in infants with severe chronic malnutrition the total exchangeable potassium, as measured by the isotope dilution method, was reduced by 25–50%/U of body weight. This was accompanied by a specific or extra loss of potassium from muscle as the ratio of potassium to noncollagen nitrogen was significantly lower than in normal muscle even after 7–10 days treatment. It is important that this severe depletion is not reflected in the serum potassium levels and treatment with potassium supplements must be continued for over 10 days. In experimentally potassium-depleted rats Cooke *et al.* (4) showed that the potassium leaving the muscle cells was partially replaced by sodium, 2 mEq of sodium passing into the cell for every 3 mEq passing out. The resulting deficiency of cations, they suggested, was compensated for by the migration of hydrogen ion into the cell with a resultant extracellular alkalosis and an intracellular acidosis. Recently Saunders *et al.* (39), using a technique based on the distribution of a nontoxic weak acid 5:5 dimethyl 2:4 oxazolidinedione between plasma and muscle, have been able to confirm this hypothesis. Whether this is also the case in severely protein-deficient muscle in human beings remains to be proved.

Magnesium has also recently had the spotlight turned upon it as it is another important ion in many vital processes. Depletion of this ion has been suspected in kwashiorkor and in malnourished Jamaican infants. Montgomery (unpublished data) has been able to demonstrate that there is a gross tissue depletion of magnesium which closely parallels that of potassium. Urine studies during recovery show an avid magnesium retention prolonged over many weeks in contrast to the relatively brief conservation of potassium and phosphorus. Blood magnesium levels as with potassium remain normal and do not reflect the gross muscle depletion that can be demonstrated in biopsy material. Clinically, however, no correlation was observed between the state of the patient and the degree of magnesium depletion, and no symptoms could be directly attributed to it.

Calcium and phosphorus deficiencies and the relationship between them, and vitamin D deficiency have been intensively studied over the last 40 years. I shall not attempt to review their role in muscle and nerve function as I am not competent to do so but the following points are worth emphasising: calcium is essential for proper neuromuscular function; it is normally found in muscle in a concentration of about 70 mg/100 g and in the serum at 10 mg/100 ml but the concentration of the diffusible form (about 6 mg/100 ml) determines the

effect on myoneural function—when its high activity is depressed; there is an intimate relationship between the biological functions of calcium and magnesium which is not fully understood but they may exert differing effects on cell permeability; finally in protein malnutrition there is a direct relationship between the amount of protein in the diet and the amount of calcium absorbed (approximately 15% on high protein diets and only 5% on low protein diets) and when the serum albumin is low the fall in protein-bound calcium is compensated for by a rise in the diffusible form (24).

CATEGORY II

Syndromes occurring in patients with a poor or marginal dietary but with no specific deficiency factor demonstrable.

A variety of neuropathic syndromes occur in different areas of the world where undernutrition is common and they were also reported for prisoner-of-war camps during the last war (5–7, 9, 20, 25, 28–33, 35–37, 45, 47). Their origin is obscure and in many cases a nutritional factor cannot easily be demonstrated. There may be an interplay of several factors such as *1*) deficiency of substances essential for nerve cell metabolism as a result of *a*) interference with the blood supply (vascular spasm or disease) *b*) failure of the body to produce them either by synthesis or from store, *c*) insufficient amounts being absorbed from the gastrointestinal tract from inadequate intake, defective bacterial synthesis or abnormalities of the intestinal mucosa, *d*) the presence of anti-enzymes or analogues in the gut or tissues: *2*) toxic factors extrinsic or intrinsic including 'allergy.'

Any combination of the above factors may be operative in a given case—hence the difficulty in determining the aetiology in different circumstances. There are 4 main presenting clinical features the relative incidence of which varies considerably from one area to another. They are *a*) pyramidal tract damage *b*) posterior column lesions *c*) retrobulbar neuropathy *d*) nerve deafness. These may occur individually or in any combination but there is no certainty that the same aetiological factor is responsible for all cases in different parts of the world or even for the different features in the same patient! Certain parts of the nervous system are more susceptible to damage than others but as we have already seen with thiamine, pyridoxine, pantothenic acid and B_{12} deficiencies, this susceptibility varies with different agents and there is as yet no satisfactory biochemical explanation for this selectivity. There are, however, many interesting possibilities. The 2 long tract neurons bearing the brunt of the damage—the upper motor neuron and the first sensory neuron for proprioception are the largest, longest and most heavily myelinated in the nervous system. They therefore carry a heavy metabolic load. Moreover, phylogenetically they are the most recent tracts of the cord. In the optic nerve the retrobulbar fibers have no neurolemmal sheath and accordingly are structurally as vulnerable as the posterior columns and lateral tracts. Also, if metabolic activity is proportional to the number of stimuli passing along an axon, the optic nerve in the tropics where light intensity is high, must be hard worked. As regards the eighth nerve lesion it is probably located in the central processes of the first sensory neurons between the spiral ganglion of the cochlea and the dorsal and ventral nuclei in the medulla. It is interesting that this portion of the nerve has no neurolemmal sheath (43). However, these observations are purely speculative and there is no experimental or other evidence as yet to give them any substance.

In Jamaica we have added a 5th feature to the 4 already listed, namely, selective lower-motor neuron damage which may arise in the anterior horn cells or anterior roots—we have not as yet had any post-mortem material from this group. In the last 8 years we have observed over 300 cases of this syndrome and we have adequate records on 210 patients. It may be of value, therefore, to give a brief clinical description of the syndrome as we see it and the significant findings are summarised in the accompanying tables.

In the majority (83%) the onset is gradual and a spastic paraplegia, sometimes with considerable loss of vibration and postural sensations, steadily develops over a period of months. Occasionally the onset is acute. A labourer in a field may be forced to give up his work within a few days. Sooner or later deterioration comes to a halt but the patient is left with some permanent disability. Paraesthesiae occur but are not common or prominent. Urinary complaints are made by half (51%) of the patients. The upper limbs are involved less frequently and to a lesser degree than the legs. Retrobulbar neuropathy (failing vision, pale optic discs and central scotomata) is found in 25%: and bilateral nerve deafness occurred in 23% of cases.

The age and sex distribution are shown in table 3.

The degree of ultimate disability varies considerably but the majority of patients are unable to follow their normal occupation and walk with great difficulty. The lower limbs are weak and spastic and exhibit the typical clonus and extensor plantar responses of pyramidal disorder. There is sometimes impairment of superficial sensation in the peripheral parts of the lower limbs but vibration and joint position sensations are often completely lost (see table 4).

One group of cases (11%), already referred to, differed from the remainder in that they showed evidence of a

TABLE 3. *Age Distribution (at Onset of Symptoms)*

10–20 Yr.	20
21–30 Yr.	36
31–40 Yr.	66
41–50 Yr.	52
51–60 Yr.	32
61–70 Yr.	4
Total	210

Sex distribution: Males, 114; Females, 96.

TABLE 4. *Clinical Features*

	Total	Percentage
Mode of onset		
Sudden	36	17
Gradual	174	83
Symptoms Include		
Pain in back	74	35
Bladder dysfunction	107	51
Neurological findings		
1) Motor. Exaggerated reflexes and increased tone. (The majority with extensor plantar reflexes.)	197	94
2) Muscle wasting. Selective wasting of hand, calf and peroneal muscles, occasionally of shoulder-girdle muscles, and 2 cases of laryngeal palsy presumably from nuclear damage	24	11
3) Sensory loss. Posterior columns	134	64
4) Optic Nerve. Retrobulbar neuropathy	57	27
5) Eighth nerve deafness	49	23

These presented in various combinations, 6 patients having all 5 features.

lower motor neuron lesion. Wasting of the muscles of the feet and calves, occasionally also of the hands and forearms, marked them out from the majority. Two of these patients had laryngeal palsies of a selective type suggesting nuclear damage. In appearance they resembled cases of peroneal muscular atrophy but there was no familial incidence and the onset, which was often moderately acute, and in adult life, suggested some other explanation. In this small group extensor plantar responses, sensory spinal ataxia and occasionally retrobulbar neuropathy and nerve deafness were also encountered. We have included them in this series of 210 patients but it may well be that further study may point to some different aetiology. So far as we know, this type of case has not been reported in any of the prisoner-of-war series nor among malnourished native populations in other parts of the world.

Routine laboratory and radiological investigations were carried out in the majority of patients and these are summarised in table 5.

Positive blood Wassermann reactions were found in 35%: this reflects the incidence of positive tests in the adult population in Jamaica. No case with a positive W.R. in the cerebrospinal fluid (CSF) is included in the series. An increase (over 60 mg %) of the protein content of the CSF was found in 18% of 190 cases submitted to lumbar puncture. Slight cellular increase was noted in the CSF in 15% and an abnormal colloidal gold curve in 24%. In all cases where myelography was carried out the results were normal. Cases with a significant degree of cervical spondylosis were excluded. A histamine-fast achlorhydria was found in 51 out of 162 patients (31%). Unfortunately the incidence of histamine-fast achlorhydria in a random sample of an equivalent age and social group is not yet known.

Dietary assessments revealed that 39% of patients were subsisting on a poor or very poor diet. The diet was fair in 18.5% and good in 24%. No dietary record was obtained in a further 18% of cases.

Post-mortem material has only been obtained from 2 cases and the detailed histopathology if only 1 has as yet been worked out. In this patient whose symptoms were numbness and rapidly developing leg weakness and who died 2½ years later from renal failure as a result of pyelonephritis, the spinal cord showed degeneration in the posterior columns and the pyramidal tracts indistinguishable from that seen in subacute combined degeneration of the cord and prior investigations had excluded B_{12} deficiency as the cause.

Strachan (49) published an account of 510 patients seen at the Kingston Public Hospital in Jamaica with a neurological syndrome which he termed 'malarial multiple peripheral neuritis.' The main features were pain, numbness and paraesthesiae of the palms and soles, dimness of vision, impairment of hearing and girdle pains. There was soreness and excoriation of the mucocutaneous junctions and evidence of polyneuritis—weakness, wasting, loss of sensation and reduction of reflexes. The gait was ataxic and the retinae showed hyperaemia and optic neuritis. The majority recovered but some died of respiratory paralysis. Again in 1918, Scott (41) described an illness which affected Jamaican sugar cane workers. It was often ushered in by stomatitis conjunctivitis and diarrhoea followed shortly by pains and paraesthesiae in the feet and legs, ataxia, deterioration of vision and deafness. Knee and ankle reflexes

TABLE 5. *Routine Investigations*

	Total	Percentage	
Blood W.R. & V.D.R.L. tests: Positive Cases with a significant degree of cervical spondylosis were excluded from the series. Myelograms in cases where indicated were negative	74	35	
C.S.F. Not examined			
Protein 0–30 mg%	20	10	
31–60 mg%	88	46	
61–100 mg%	68	36	of
100+ mg% (max. 150)	28	15	C.S.Fs.
	6	3	examined
Cells greater than 4/c. mm (max. 35)	29	15	
Colloidal gold curves: Paretic	31	16	
Luetic	15	8	
The W.R. & V.D.R.L. tests were negative in all cases as those with positive reactions were excluded from the series			
Acidity of gastric juice			
Histamine-fast achlorhydria	51	24	
Free HCl after histamine	29	14	
Free HCl present	82	39	
No record	48	23	
Dietary assessment			
Very poor	30	14	
Poor	52	25	
Fair	39	18.5	
Good	39	18.5	
Very good	12	6	
No record	38	18	

were lost and the plantar responses remained normal. The only objective sensory loss was some impairment of temperature sense and position sense was said to be normal. Vibration sense was not recorded. Some patients died in 4–6 weeks and in many there were residual neurological defects. These were mainly loss of vision, deafness and ataxia. The latter is hard to explain if position sense was normal and there is nothing in the clinical reports to suggest it was cerebellar in type—perhaps residual muscle weakness was responsible. At autopsy degeneration in the fibres of olfactory, optic and auditory nerves was found. The pyramidal tracts largely escaped but considerable degeneration was found in the posterior root ganglia and in the posterior and spino-cerebellar tracts of the spinal cord. Scott did not think the disease was of nutritional origin but considered a toxaemia more likely. Both of these accounts point to a sensory type of neuropathy with a tendency to residual ataxia and impairment of vision and hearing. Since then the numerous reports already referred to, of similar or related syndromes from different parts of the world have accumulated. These are drawn from three broad groups *1*) undernourished populations in tropical countries—Africa, the Far East and Jamaica; *2*) prisoners of war in the Middle and Far East (1941–45); and *3*) the besieged population of Madrid during the Spanish Civil War (1936–39). These disorders are not seen in Europe (with the exception of Spain) and North America and did not occur in the European concentration camps of the last war.

The circumstantial evidence therefore suggests that malnutrition, using this term in its broadest sense, is mainly responsible. Many of our Jamaican patients, however, appeared to be in good health, and their histories indicated an adequate and balanced diet. Many were employed in heavy labour. Frank signs of known vitamin deficiency were not encountered. In other parts of the world evidence of malnutrition in these syndromes has often been obvious and it has usually taken the form of the mucocutaneous lesions of vitamin B_2 deficiency. This was also true of the earlier Jamaican cases of Strachan (49) and Scott (41), and it is to be noted that these differed in many respects from the present series—notably in the absence of pyramidal tract involvement—and resembled more closely the syndromes described elsewhere. In the prisoner of war camps, on the other hand, the neurological features often developed against a background of apparently fair general health despite loss of weight and energy. In other words, we must not necessarily expect to find the classical signs of vitamin deficiency. Since Addison's time we have known that the patient with pernicious anaemia or subacute combined degeneration may appear well-nourished. His deficiency manifests itself in a very selective way; and from the neurological point of view vitamin B_{12} deficiency more closely resembles the syndromes under discussion than any other common disease of the nervous system.

A normal bone marrow was found in over 20 of the cases with a histamine-fast achlorhydria, and the great majority of the patients had normal blood pictures. This, and a normal Schilling test in 4 typical cases, make it very unlikely that B_{12}-deficiency is an important aetiological factor. In addition, studies of blood pyruvate and glutathione have proved negative, although cases in the early acute stage were not available for these estimations. A megaloblastic marrow was found in 2 women aged 22 and 29 years at the time of onset of the neurological symptoms, one of whom developed bilateral eighth nerve deafness. The diet of one was fair and her marrow rapidly returned to normal with B_{12} taken orally. The other patient's diet was very poor and her blood picture returned to normal with hospital diet alone. In neither was there any improvement in the neurological symptoms. In the circumstances they cannot be regarded as true cases of pernicious anaemia with subacute combined degeneration. Subacute combined degeneration has, however, been described in patients assumed to have B_{12} deficiency from inadequate intake, and this might be the explanation in these 2 patients (56).

Classical pernicious anemia has been regarded as rare in Jamaica, and only 9 cases (7 proved by the Schilling test) have been recognised at the University College Hospital out of 43,000 in-patients admitted over the 8 years the hospital has been operating, and 4 of these have had signs of subacute combined degeneration of the spinal cord. Of these 9, however, 7 have been diagnosed in the last 2 years and it is possible that more careful screening of haematological disorders may reveal a considerably higher incidence than previously believed.

Degazon (8) has reviewed 298 cases of 'tropical amblyopia' seen in the Kingston Public Hospital, Jamaica, in the period 1948–55. Nine patients suffered from nerve deafness; none had evidence of spinal cord disturbance. Males and females were equally affected and the majority of cases occurred between the ages of 20 and 50 years. The highest incidence among males occurred in the 20–29 age group, and among females in the 40–49 age group. The occupational incidence represented a normal cross-section of the ophthalmological out-patient population of the hospital. The clinical features—dimness of vision, pallor of the optic discs, and central and paracentral scotomata—corresponded closely to those described elsewhere in the world and among the prisoners of war. Treatment with substances of the vitamin B complex appeared to have beneficial results in early cases. Nevertheless, Degazon (8) found little evidence of malnutrition and preferred to speak of 'tropical amblyopia' rather than 'nutritional retrobulbar neuropathy.' He suggested that hard physical labour in males, uterine blood loss in the females and the continuous exposure on the retina to bright sunlight might be factors of some importance.

Although we agree that many factors may contribute to tropical amblyopia, it appears that there may be 1 specific requirement, and we suggest that an absence

or overt signs of general malnutrition must not be taken to mean that a specific deficiency is not in operation. Surely, the history of vitamin B_{12} deficiency has taught that lesson.

Of importance in these Jamaican cases is the apparently high incidence of serological tests for syphilis and yaws.

We have excluded from our series all cases showing serological abnormality in the cerebrospinal fluid. The incidence of a positive blood Wassermann or Kahn reaction in Jamaican adults varies from 12 to 41% in different parishes, with averages of 23% for farm labourers and 40% for male industrial workers (19). There is no means of differentiating yaws and syphilis by serological tests, but it is thought that latent yaws, which is endemic in Jamaica, may be responsible for a large number of positive serological findings, particularly in the hilly country areas where clinical yaws used to be common.

Thus, a positive blood Wassermann or V.D.R.L. test in 35% of our 210 cases is no more than the average for the population, and 65% had no clinical or serological evidence of syphilis, except for 2 patients with negative serology, one of whom had aortic incompetence and a dilated ascending aorta, and the other a fusiform dilation of the descending aorta. A very high proportion of the patients were born and brought up in the country—a much higher percentage than in all other chronic sick in the medical wards of this hospital. It is of interest that yaws was until recently common in the same areas, and is not found in the town-bred population; a few patients in this series with negative serology gave a history of yaws. At the same time, the country population has much readier access to a wide variety of bush teas. Yaws and syphilis might be capable of producing such neurological damage, and yaws is also prevalent in other countries where unusual neurological syndromes occur. However, neurological sequelae of yaws have never been recognised (21), nor do these disorders appear in the accepted clinical patterns of neurosyphilis.

Heredity does not seem to play a part in the Jamaican syndrome. Cases arise in all parts of the island with no evidence of interbreeding, and only once has a familial incidence been observed, in 2 sisters.

An interesting feature is the selective muscle wasting in 24 patients, indicating damage to the anterior horn cells or anterior roots. There was no evidence of local disease of the spinal column such as spondylosis, and no abnormality in the myelogram of these patients. This selective lesion has not to our knowledge been described previously, and its cause is as mysterious as the other features of the syndrome.

Comparison of the Jamaican cases with those recently reported from West Africa (32) shows a very much higher incidence of upper motor neurone damage, which is frequently the earliest and only feature in our patients, occurring in 94% of our cases, compared to 3% in West Africa. A predominantly spastic syndrome was seen in prisoners of war in the Far East but not the Middle East, and has recently been reported in Eritrea (13).

Other differences in our cases include the very low incidence of paraesthesiae which was an initial symptom in 99% of Money's cases. Differences of this kind suggest that the syndromes in different parts of the world may well be due to a variety of factors producing closely related lesions. There seems little doubt that the neurological picture in Nigeria and elsewhere has been complicated by the presence of thiamine deficiency, which is evident in few, if any of our cases.

In addition to the possibilities discussed above, consideration has been given to the questions of autoimmunity, toxic fractions in cooking oils, trace metal deficiency, or toxicity due to selenium, molybdenum or cyanide, which may variably be present in excess in bushes, grasses, yams and cassava.

Much more detailed information is required of the environment in which these disorders occur in different areas of the world. Until similarities and differences are established, and there is some clue on which to base experimental work, speculation as to the exact aetiology must continue to range over a wide field.

CATEGORY III

Syndromes where there is a known toxic factor but undernutrition may play a contributory part. Of these the only one I shall discuss is lathyrism.

This neurological disorder has been recognised since biblical times and Stockman (48) suggests that it was known to Hippocrates. Its possible relationship to the ingestion of legumes was first noted by Ramazzini who described an outbreak in Modena in 1690. A high incidence of nearly 7% in certain areas of India was reported nearly 100 years ago, Irving 1868 (26) and Young (57) gives a similar figure for field studies in several Indian villages. The lathyrus family grow readily even under adverse conditions and when grain crops fail they may be consumed in large quantities. There is a striking male preponderance particularly in young active men probably because their total consumption is considerably higher than women or older people and hard physical work may contribute.

There have been different views on the specific aetiological factors and conflicting experimental work ranging from claims that a virus with generally poor nutrition is responsible through Vitamin A- or B-deficiencies to specific toxic agents such as a glucoside, an alkaloid (divicin), phytates and amines (50). Within recent years however, nitrile compounds have been isolated from the lathryus family (40) and these have been shown to produce damage to mesenchymal and neural tissues in experimental animals. The nitrile compound responsible for the mesenchymal damage—particular skeletal and vascular lesions—is beta-aminopropionitile (BAPN) (38) and there is evidence that these lesions result from interference with the production of cement substance both in the blood vessels and grow-

ing bone and the resemblance to scurvy is striking but the collagen fibres are unaffected. The cement substance in cartilage is probably chondroitin sulphate but not much is known of the cement substance elsewhere (1). The lesions can be precipitated by giving a monoamine oxidase inhibitor (iproniazid) suggesting that beta-aminopropionitile is normally detoxicated by monoamine oxidase as giving iproniazid alone does not produce the lesions, and that the harmful action of BAPN is due to metabolic interference through its amino group (27). Neither pyridoxin nor pantothenic acid protects against this action. BAPN is found in lathyrus odoratus and not in the group of the lathyrus family which have been incriminated in human lathyrism and there is some evidence that the nitrile compound responsible for the nuerological lesions is iminodipropionitrile (42). Monoamine oxidase is an important enzyme in neurochemical transmission as it deaminates and destroys epinephrine, tyrosine, dopamine and particularly serotonin. This latter is present in particularly high concentrations in the central nervous system and the affinity of monoamine oxidase for it is greater than that for other catecholamines. If large amounts of these nitrile compounds are ingested and if there are inadequate amounts of monoaminic oxidase to ditoxicate them fully, metabolic interference in the neurochemical activity of central nervous tissue may result and lead eventually to structural damage. In addition many of the enzymes of neural tissue are pyridoxin dependent particularly the decarboxylases which are essential for the metabolism of glutamine, the only substance that readily passes the blood brain barrier and is essential for the replenishment of substrates for the tricarboxylic acid cycle in central nervous tissue (3). A low pyridoxin intake may therefore precipitate or aggravate the neural damage from these nitrile compounds or other as yet unrecognised toxic factors in neuropathies of uncertain origin.

I have stepped well out of my depth with these biochemical speculations but they may at least provoke discussion.

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Nutritional aspects of cirrhosis and carcinoma of the liver

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THE TITLE OF THIS PRESENTATION, suggested by the programme committee, and the recommendations added by the Moderator, made us decide that we should try and cover: *a*) a brief outline of work done in this field (more specifically in Jamaica), giving the present state of affairs; *b*) suggestions to extend our observations through a centrally organised world-wide effort.

A discussion of nutritional aspects might conceivably include substances taken by the various peoples of the world, such as alcoholic beverages or other potations. These may act upon the liver cells either directly or via metabolic pathways and so pave the way for other noxious substances (cirrhosiogens or carcinogens), such as viruses, chemical substances, etc.

A brief review like this, inevitably the result of a drastic sifting of literature reports, is necessarily biased, for good as well as bad reasons. We apologise to all the eminent students in this field whom we could not mention in the space available.

NUTRITIONAL ASPECTS OF CIRRHOSIS OF THE LIVER

The bases for the concept of under- or malnutrition as a factor in liver diseases have been firstly, the inordinately high incidence of liver disease in man in underprivileged areas of the world and secondly the outcome of experimental work in animals.

While earlier investigators, among whom authorities like Mallory and Eppinger, quoted by Himsworth, (49), tended to separate fatty change in the liver from fibrosis in that organ, the experimentalists demonstrated, mainly in the rat, a firm connection between the 2 processes. The reviews by György (37, 38, 40) summarize most of the relevant experimental work. The interrelation between various dietary factors involved in experimental liver damage may be conveniently shown as in table 1.

Experimental dietary necrosis develops in rats on diets deficient in cystine, vitamin E and factor III. This may in fact be reduced to a deficiency of 2 factors: Vitamin E and selenium, present in factor III and in small traces also contaminating cystine. Schwarz (73), Schwarz *et al.* (74). It has no strictly comparable equivalent in man, possibly because of the rare simultaneous absence from the diet of all factors involved. It has, however, been most valuable as an experimental model of hepatic injury.

Experimental dietary fatty change and cirrhosis of the liver may be produced in rats on a diet deficient in lipotropic factors, in particular choline and its precursors: methionine, betaine. Supplements like vitamin B_{12} exert their beneficial effect probably through their choline-sparing action. The temporary beneficial effect of antibiotics on dietary necrosis and cirrhosis György and Stokes (39), György (40, 41) is probably through better or selective utilization of amino acids, i.e. through their effect on protein.

The fatty change is centrilobular in the absence of choline or its precursors, but peripheral when inadequate proteins (unbalanced amino acids) are supplied in the presence of choline, Shils and Stewart (75, 76), Best *et al.* (4). Ramalingaswami (personal communication) produced at will fat in centrilobular (diet low in protein, high in fat, deficient in choline) or peripheral location (diet low in protein, low in fat, deficient in choline). Only if choline or its precursors are absent will fibrosis follow the fatty change. The pathogenesis of this, according to Hartroft (44, 45), takes place via rupture of the membranes of the grossly distended fatty cells. Other workers György and Goldblatt (35, 36), Handler and Dubin (43), Koch-Weser *et al.* (53), Bras *et al.* (9) placed emphasis on cellular necrosis, irrespective of fat overload and/or rupture of cell membranes.

A further consideration enters into the dietary experiments, i.e. calories, see e.g. Mulford and Griffith (56), Naftalin (57, 58) and Ramalingaswami *et al.* (67); the development of fatty change and cirrhosis being facilitated when calories are increased while at the same time the amount of lipotropic or other cirrhosis preventing factors remains constant.

Sriramachari (78) showed that severe protein de-

[1] Dr. S. E. H. Brooks and Dr. D. C. Watler are co-authors of the data produced in this communication.

TABLE 1. *Effect of Some Dietary Factors in Experimental Liver Injury (Adapted From György 1957)*

	Necrosis	Cirrhosis
Protein	Beneficial	Beneficial
Methionine	Beneficial	Beneficial
Cystine	Beneficial	Injurious
Choline	No effect or injurious	Beneficial
Vitamin E	Beneficial	No effect
Dietary fat	No effect or injurious	Injurious
Vitamin B_{12}	No effect	Beneficial
Factor III	Beneficial	Unknown

ficiency evidently could not sustain a continued fibrosis and caused its reversal. The latter was prevented when protein was added to the diet.

The results of these experiments naturally influenced the interpretation of clinical observations: Kwashiorkor, which in most patients is characterised by a substantial fatty change of the liver (up to 30–50% of the wet weight of the organ) Waterlow and Weisz (93), Waterlow, Bras and DePass (94), developed on the basis of protein deficient nutrition, has been under grave suspicion of being the precursor of fibrosis and cirrhosis.

Observers in Africa, Vint (88), Davies (20), Trowell *et al.* (86) noted an apparent association between kwashiorkor and fibrosis, while Trowell (87) further indicated that stellate fibrosis may, in addition, develop in persons on a marginal diet; Wahi (90) also inferred that subclinical kwashiorkor may produce hepatic fibrosis. This, then, would indicate a nutritional or metabolic injury to hepatic cells concomitant with or followed by a stimulus inducing proliferation of the connective tissue (Gillman and Gillman, (30)). Investigations into the nature of the cellular injury—apart from morphology—have also included studies of several biochemical aspects, notably the protein content of the liver and its enzymatic activity (see Waterlow (97) for an informative review). It would appear from this work that the significant result of a protein deficient diet is the derangement of the relationships in multienzyme systems rather than the loss of any single enzyme (Waterlow, (96)).

Work in Jamaica suggests that the fatty change in kwashiorkor is a kind of epiphenomenon: the degree of infiltration seems to bear very little relation to the clinical severity of the disease, Waterlow, (92), Bras *et al.* (67), and it causes very little disturbance in tests of liver function. It should be recalled that Davies (19, 20) has always maintained that the pancreas occupies a pivotal position, in that the protein deficiency renders it unable to produce its enzymes; this in turn causes other changes in the body. Gillman and Gilberts work (32) further demonstrates beautifully the intimate relationship that may exist between the dietary and hormonal factors. Functional and structural changes in endocrine glands were also reported from India by Gopalan (34) and by Wahi *et al.* (89).

The view that kwashiorkor was followed by fibrosis and cirrhosis was widely held 10 years ago. It has now, however, almost universally yielded to the more cautious opinion that 'it is as yet not proven that kwashiorkor is a precursor of fibrosis' (Waterlow and Bras (95)). This view is based upon evidence which includes the following: i) Although cirrhosis of the liver is commoner in some areas where kwashiorkor is common, it is also a fact that in other areas where kwashiorkor is common, cirrhosis may be uncommon (Report Subcommittee of Kampala Conference, (51)).

As the fatty liver of kwashiorkor is the liver lesions for which a dietary origin can be claimed with most confidence—albeit that we must accept the fact that it is not a specific change and that its mode of production is still largely unknown—it is important that a world-wide effort be launched to establish the presence or absence of a causal relationship between kwashiorkor and cirrhosis. In this connection one must not overlook the suggestion made by Davies (21) and Platt (62): They have pointed out that in underdeveloped communities food supplies tend to be seasonal, with alternating cycles of shortage and plenty. It is possible that this kind of recurrent stimulus might have a more damaging effect on the liver than a steady state of chronic deficiency. Also, in the past, lasting effects of faulty nutrition in early life have been largely ignored or not sufficiently taken into consideration (György (41)). That such effects may become apparent in later life has been well demonstrated by Best and his colleagues on the effect of early choline deficiency in rats. There was a significant high incidence of hypertension and nephrosclerosis in rats which had received a choline deficient diet for a short time in the post-weaning period and then intermittently.

Dible (24, 25) was among the first to express doubt in the face of impressive experimental data on rats—that fibrosis is a common sequel to fatty change. Van der Sar (71) reported kwashiorkor but no fibrosis from Curacao. Brock (11, 12) did so at the Gambia and Kampala Conferences in 1952 (29) and 1957 (51), respectively and we reported in 1953 and 1957 at the Jamaica (92) and Cali Conferences (unpublished data) a lack of fibrosis in a follow-up of kwashiorkor cases, and this has been confirmed by our observations to date. Similar findings have been reported from other countries, notably Asia (Ramalingaswami *et al.* (66), Sriramachari *et al.* (77), and the Belgian Congo (DeMaeyer (23)). The evidence then, is that exposure to a deficient diet, although predisposing to kwashiorkor, does not necessarily predispose to cirrhosis. If this is accepted, the term nutritional or tropical cirrhosis becomes undesirable.

ii) Walters and Waterlow (91) demonstrated in the Gambia fibrosis in children not preceded by fatty change. Malaria (superimposed upon malnutrition) was incriminated on convincing circumstantial evidence. This supported certain facts and hypotheses, i.e. that parasites, bacterial and viral diseases may constitute noxious agents, made all the more potent in a liver rendered vulnerable by mal- or undernutrition. The noxious nature of some of these, notably virus hepatitis

and schistosomiasis, is now well documented. Findlay (28) reported that although the morbidity rate for infectious hepatitis was low in Africans, the mortality rates were much higher. This could imply that the development of post-hepatitic cirrhosis in those Africans was also higher, i.e. this virus disease ravaging the preconditioned liver of the underprivileged oftener leads to cirrhosis than would be the case when Europeans are affected. Reports from West Africa (Bergeret and Roulet (3), Charmot *et al.* (15), Camain (13), and from Indonesia (Straub and Schaberg (83) Pang (60)) substantiate this.

iii) Our own work in the West Indies, Bras *et al.* (5) Stuart and Bras (84), Bras and Hill (6), Bras *et al.* (8) demonstrated liver cirrhosis in children and adults resulting from ingestion of bush teas (Crotalaria fulva). While extensive use of such herbs is only made by those not readily seeking medical advice—i.e. the poorer section of the population—the liver damage is probably not related to the nutritional background. In our experiments the toxicity of these plants could not be prevented by feeding rats adequate amounts of a protein of high biological value. These observations indicate the possibility that other toxins (alkaloids or other)—not as spectacular as Crotalaria in their cause and effect—may well be cirrhosiogenic, irrespective of a protein deficient background.

For the Indian childhood cirrhosis it has been claimed repeatedly that it occurs among the higher social classes. Vegetarianism among these groups confuses the dietary issue somewhat, but it would appear to us that—like in veno-occlusive disease (V.O.D.)—undernutrition is not a requisite as a background to Indian childhood cirrhosis (Achar and Chacko (1), Srivastava and Aikat (79), Mukerjee *et al.* (55), Indian Council Med. Res. (50), Amarjit Singh *et al.* (2)).

Denial of a direct causal relationship in man between protein deficiency with fatty change-cirrhosis does not necessarily militate against the overwhelming evidence of such a sequence in the experimental animal. The time and intensity of fatty change, such as is present in the experimental rat, are practically nonexistent in man. Further, lipotropic substances so effective in the experimental animal are of no use in the treatment of fatty liver disease.

It would appear, then, that we have to look upon underprivileged man as an individual who goes around in a world which denies him a properly balanced nutrition in sufficient quantity, who is being denied non-essentials such as a car, a wireless set and a daily newspaper along with essentials which we do not know, who further is exposed to unsanitary conditions, to misguided treatment by witch doctors, and presumably a host of other potentially noxious factors. Further, interdependent enzymatic, nutritional, metabolic and hormonal factors may all be concerned (Platt and Heard (63)). This then assumes the proportions of a problem with as yet uncountable unknown variables and it is small wonder that the path to lead him out of this wilderness—apart from the seemingly impossible raising of his economic level—has yet to be indicated.

Observations that the U. S. Negro is not more liable to acquire cirrhosis than his white compatriot have effectively dealt with the possibility that racial factors are at the root of the problem (Dorn (26)). On the other hand, immigrants into the U. S. from Mexico and the Far East carry with them the risk of developing cirrhosis (Steiner (81)). It is interesting in this respect to recall the observations of Senecal and Payet (74*a*) in Dakar that the African child, having a distribution of serum proteins in its first year identical to that of its white counterpart, is different already at 4 years of age, when he has developed an inversed albumin/globulin ratio due to a large increase in gamma globulines. Payet (61) further suggests the possibility that the increased gamma globulin, by virtue of providing anti-immune reactions gives rise to a continuous self-maintained disease. The inversion of the albumin/globulin ratio seems practically irreversible, as it was still present in young adults who had for 6 to 9 years been living in better economic conditions, i.e. along with African-born whites who had a normal protein ratio.

In this respect we might refer to studies by Ross (69), who placed rats on a variety of life-long nutritional regimens. Profound changes in patterns of liver enzymes resulted and the life span of the rats was significantly affected. As we were privileged to study these rats histopathologically, we know that the incidence of disease and of neoplasms was significantly different among the various groups and apparently for no other possible reason than just a different (life-long) diet (unpublished data). These experiments by providing an experimental model will, we submit, at some time assist in understanding incidences of a wide variety of conditions, e.g. the paucity of hypertension and myocardial infarction in Uganda (Trowel (87)) and rarity of myocardial infarction in Jamaica, even though atheromatosis of the aorta is of the same intensity and extent as in the U. S. (Robertson, (68)), and many others.

What suggestions might be made to fill the gaps in our knowledge?

a) It would appear that 'base-line studies' are needed. Surely, if in Dakar the albumin/globulin ratio is irreversibly altered as early as the fourth year of life, it is indicated that other biochemical anthropometric studies be made.

b) Differences in nomenclature for cirrhosis still confuse many reports, although the Havana and Kampala Conferences laid down clear-cut criteria. The Kampala recommendations that an Atlas should be prepared, illustrating various liver lesions, should be implemented. To be effective however, it should be distributed—at no cost—to investigators in all parts of the world. The national governments may well be asked to contribute to this.

c) Study groups in various parts of the world, already

concerned with these problems, might be asked to investigate aspects of malnutrition along certain clear-cut lines, statistically sound and yet practicable in relatively remote areas. This might include the natural history of kwashiorkor in mild and severe cases, its epidemiology and sequelae, requiring the completion of a separate questionnaire for each patient. Cancer Registries in various parts of the world have succeeded in accumulating statistically valuable material in this way, and it should be possible to do this for noncancerous diseases. Data obtained from cross-sectional studies through the population could provide additional information.

d) Occasional visits to the various territories by greatly respected authorities should prove a stimulus and further ensure uniformity.

e) The study of the substructure of liver cells with the electron microscope is now providing us with more anatomical details but as yet has not advanced our understanding of malnutrition (Camain *et al.* (14)). Efforts should continue to define the functional and metabolic changes in the liver that precede structural changes, and that make it vulnerable to injury from every quarter. Until this has been done, the 'conditioning' that has been postulated by almost everyone remains a hypothesis.

NUTRITIONAL ASPECTS OF CARCINOMA OF THE LIVER

For obvious reasons I shall restrict my remarks to primary hepatocellular carcinoma. The present International Classification of Diseases, incidentally, includes under No. 155.0 both hepatocellular and cholangiocellular carcinoma. In many statistics, therefore, a combined figure is given for both, whereas geographical pathological studies clearly point at remarkable differences for which it would be important that these 2 types of carcinoma should be separated in statistical data. We recommend, therefore, that the International Classification of Diseases be amended in this respect (e.g. to read 155^a hepatocellular carcinoma, 155^b cholangiocellular carcinoma, 155^c mixed types). Even if, as some accept, all 3 types originate in the liver cell (Gillman and Payet (31), Roulet (70)), such a distinction in well circumscribed groups should be useful.

The types of hepatocellular cancer appear to be the same everywhere in the world and, apart from the statistical and aetiological aspects, it is the same disease anywhere. There is, however, some confusion regarding nomenclature. As with cirrhosis, an atlas would go far to clear up misinterpretation.

"Circumstantial evidence and logic, linking nutritional factors and hepatic cancer, are impressive" (Tannenbaum (85)). This is a view for which support could be sought as follows:

a) There is a tendency for a liver with cirrhosis (particularly of the macronodular type) to develop cancer. From Uganda Davies (22) reported cancers particularly following chronic Laennec's cirrhosis, but Steiner and Davies (82) consider these cirrhosis cases probably viral in aetiology. From South Africa Higginson (47) reported post-hepatitic cirrhosis to outnumber by far portal cirrhosis. Davies concedes that carcinoma develops in the damaged liver but is becoming "less and less convinced that carcinoma arises from cirrhosis" (Davies (22)). It is interesting to note here the development of carcinoma in cirrhosis following veno-occlusive disease in Jamaica.

In several places in the world there is a remarkably similar figure of approximately 16% of all cirrhotics having hepatocellular carcinoma. Dible's figures for Britain (25), those of Roulet for Switzerland (70), Davies figures for Uganda and ours from Jamaica (10) are of this order. In Johannesburg, Portugese East Africa and Indonesia the figure is higher and is around 40%, while in the United States it is lower (McDonald (54), Steiner (81)), possibly because one out of every 4 deaths from cirrhosis of the liver in males is associated with alcoholism (Dorn (26)); the course of the disease thus may in the United States be a more rapid one, giving as it were no time for carcinoma to develop.

As discussed above, the link between cirrhosis and undernutrition is open to several considerations and reservations, which then also apply to the link undernutrition/carcinoma. Furthermore, undoubtedly cancer of the liver also develops in the noncirrhotic liver, although such a liver might conceivably have had fibrosis, earlier in life (Hartroft (46)).

b) Induced hepatic carcinoma can be modified by dietary means. A butter-yellow induced carcinoma in the rat's liver can be interfered with when the host's biology is altered through a change of diet, and also by producing endocrine changes (Tannenbaum, (85)). A most interesting finding here was that riboflavin proved to be a coenzyme necessary in systems which metabolically inactivate azo-dyes, thus interfering with their carcinogenic activity. Higher levels of dietary riboflavin therefore reduce tumour formation.

c) In man, other carcinomas, apart from those in the liver, are known or suspected to be linked with dietary factors, e.g. thyroid carcinoma in individuals with nodular goiter as seen in iodine deficiency. Also, there appears to be a relationship between carcinomas of the oral cavity, pharynx and upper oesophagus, and Plummer-Vinson syndrome in females; the syndrome is thought to be caused by a diet low in iron and vitamins.

d) Choline deficiency, when prolonged, gives rise to liver cancer in experimental animals.

e) Ethionine feeding produced hepatic lesions and tumours, presumably either through metabolic antagonism of methionine or because of its toxicity (Popper and Schaffner (64)).

f) Substances, present in food, could well account for some tumour development since e.g. alkaloids (Schoental *et al.* (72a)) and chili peppers (Hoch-Ligetti (49a)) did produce such tumours in the rats. Some investigators stressed the importance of a qualita-

tively poor ration as an important concomitant factor in these experiments, but our own work with alkaloids (referred to under cirrhosis) infers that such a view may be too restricted. Schoental's work (72) further indicates that ingestion of toxins through mother's milk must be borne in mind as a possibility.

What, then, are the present thoughts about the causation of primary cancer of the liver?

In the development of liver carcinoma, liver cirrhosis is not a condition sine qua non. However, since a statistical association exists between the 2 liver diseases, cirrhosis and cancer, one must consider that: i) they are caused by the same factor(s); ii) the former is causing the latter; iii) the former is modifying the liver so that causative factors inducing carcinoma become more effective (this is the proposition postulated by many workers, explicitly or implicitly); iv) that the association is coincidental.

Infectious hepatitis, per se, is unlikely to be the immediate cause of cancer, as epidemics of this disease in nontropical parts of the world have not been followed by a rise in incidence of liver carcinoma (Clemmesen and Nielsen (17)). However, the possibility cannot be excluded that the virus participates in promoting liver cancer in some particular metabolic context (Gillman and Payet (31)).

TABLE 2. *Incidence of Liver Cancer in Jamaica (Kingston and St. Andrew) Occurring in 1958–1959 Compared with the Expected Number from other Populations of Identical Size, Sex and Age*

Age Group	Mean Population at Risk in Jamaica (1958–1959)	No. of Cases	Expected No. of Cases in Similar Population	
			Bantu (1953–1954)*	Denmark (1943–1947)†
		Males		
5–14	31,043	0	1.10	0
15–24	28,667	0	1.28	0
25–34	27,821	3	8.91	0
35–44	24,426	3	17.22	0
45–54	12,532	3	7.57	0.15
55–64	6,473	1	12.42	0.24
65–74	2,998	0	8.94	0.25
75+	1,307	0	0	0.16
Total	135,267	10	57.44	0.80
		Females		
5–14	33,937	0	0	0
15–24	44,601	0	1.61	0
25–34	42,654	1	1.40	0
35–44	28,347	0	2.94	0
45–54	17,435	0	5.46	0.34
55–64	6,069	0	13.57	0.09
65–74	5,135	0	8.43	0.14
75 +	2,650	0	6.94	0.16
Total	183,828	1	40.35	0.73

*Oetle and Higginson (59). † Clemmesen and Nielsen (16).

TABLE 3. *Occurrence of Primary Carcinoma of the Liver in Cirrhosis (1833 Consecutive Autopsies, University College Hospital, Jamaica)*

Type of Cirrhosis	No. With Cirrhosis	Number With Carcinoma			Percentage With Carcinoma
		Hepatocellular	Cholangiocarcinoma	Total	
Laennec's	24	4		4	16.7
Post necrotic	27	5		5	18.3
Post V.O.D.	28	2		2	7.1
Biliary	1				
Undetermined	9	3	1	4	44.4
Total	89	14 (12 M) (2 F)	1 (1 F)	15 (12 M) (3 F)	16.9

Parasitic infestation must be considered as possibly important concomitant factors, e.g. schistosomiasis for Mozambique (Prates (65)) further malaria and nematodes for most parts of the tropics.

If consumption of local medicinal herbs plays a role it is not likely that the same carcinogen operates in geographical areas so far apart as those in which liver carcinoma has an inordinately high incidence. In this respect it is interesting to extend Feldman's (27) and Davies' studies (22) who reported the occurrence of spontaneous liver carcinoma in animals in different geographical areas.

In animals, diet alone can produce liver carcinoma and diet therefore may at least be a contributory factor in man. The metabolic background has been demonstrated to be extremely important in the butter-yellow experiments quoted above, while Ross' work referred to earlier, under cirrhosis, would again confirm this. In the U. S., however, (Steiner (81)) there is no evidence of undernutrition playing any role at all, although, as he says: "in contrast to absence of malnutrition of the body as a whole there may be that of the liver itself. It is easy to imagine nutritional deficiencies in some liver cells in relation to severe fatty changes, cirrhosis and circulatory impairment."

These wide gaps in our knowledge of the disease would suggest the following: *a*) that the circulation of an atlas, as for cirrhosis, should prove an important tool in providing a common nomenclature, well understood by all. *b*) In addition to kwashiorkor studies as outlined earlier, a cancer registry seems greatly needed in several tropical areas. Gillman and Payet (31) elaborated on the necessity for this, indicating at the same time their valid criticisms with regard to many of the incidence figures as given in the literature. We operate such a registry in Jamaica (since January 1, 1958) in which all patients residing in a predominantly urban area (from hospitals and general practitioners) are included. The figures are thus of an accuracy as one would necessarily require for a comparative study. Comparison with figures obtained in cancer registries elsewhere is shown in table 2. Table 3

TABLE 4. *Incidence of Primary Carcinoma of the Liver and its Relationship to Cirrhosis in Various Countries*

Observations	Jamaica	Switzerland (Roulet (70))	England (Dible, (25))	U. S. A. (Edmondson and Steiner, 1954)	Uganda (Davies, (22))	S. African Bantu (Oetle and Higginson, (59))
1. Total No. of autopsies	1,833	23,470	7,217	48,900		
2. Cases of hepatocellular carcinoma	19	93	22	75	80	80
3. Cases of cholangiocarcinoma	4	16	17	25	17	4
4. Mixed cases or uncertain type			2			4
5. Incidence of hepatocellular cas. in all autopsies	1.0%	0.4%	0.3%	0.15%	—	—
6. Incidence of cholangiocas, in all autopsies	0.2%	0.07%	0.2%	0.05%	—	—
7. Ratio of hepatocellular cas:cholangio ca.	4.75:1 (autopsies) 9:1 (standard population)	5:1 (autopsies)	1.3:1 (autopsies)	3:1 (autopsies)	4.7:1 (autopsies)	20:1 (autopsies & biopsies)
8. Incidence of hepatocellular ca. in cirrhosis	15.7%	15.8%	16.5%	4–8%	—	—
9. Incidence of cirrhosis in hepatocellular ca.	86% males 40% females	86% males 36% females	82% both sexes	90% males 50% females	92% males 62.5% females	80.8% both sexes
10. Ratio of males to females in hepatocellular ca.	3:1 (autopsies) 8:1 (standard population)	5.6:1 (autopsies)	4.5:1 (autopsies)	4:1 (autopsies)	9:1 (autopsies)	10:1 (autopsies & biopsies)
11. Incidence of cirrhosis in cholangio ca. (both sexes)	25%	18.8%	18.0%	23.5%	47.0%	33.3%
12. Ratio of males to females in cholangio ca. (in autopsies)	1:1	3:1	0.7:1	1:1	3.25:1	1:1

TABLE 5. *Weights of Kingston School Boys*

Age	7	8	9	10	11	12	13
Boys: weight (lb.)							
1935–37	45.4	49.3	55.4	61.8	66.3	71.6	76.7
1938–40	46.0	50.8	56.1	61.1	66.8	72.2	79.5
1941–43	45.4	53.5	58.5	64.3	70.5	77.2	81.3

provides some relevant data on cirrhosis and carcinoma of the liver, while table 4 compares some geographical pathological data.

In view of this, the following nutritional background of the Jamaican population might be of interest.

A pilot nutritional survey was carried out in 5 Jamaican areas during 1957 (Standard (80)). It was noted that 72% of all households surveyed used bush teas and 60% of these gave it to infants in the first year of life. Calorie and protein intakes were low. Liver enlargement one finger's breadth below the costal margin was found in 10% of the children varying from 0% in 1 area (Pedro Plains) to 30% in another (Rock).

The majority of weights of children were below English averages, but the heights approximated the English averages. This fact has also been previously remarked upon by Cumper (1960) (personal communication), who analysed data on 5,000 Kingston schoolchildren. He pointed out the possibility of racial factors affecting body form. However, his figures (table 5) indicate improvement among the Kingston children over a 9-year period. These figures are now 17 years old and possibly further improvement has occurred.

More detailed study has been carried out by Gourlay (1960) (personal communication) and his co-workers on a rural population of 6,000 people. He found a surprisingly low caloric intake among apparently healthy adults. Some other available data on dietary surveys in Jamaica is presented in table 6. In general therefore, on the basis of somewhat limited data, it appears that many Jamaicans are on a low protein, low caloric diet.

Figures for cirrhosis were quoted in table 3: 30% of these were of the Laennec, 30% of the post-necrotic and 30% of the post-V.O.D. type. Parasites in Jamaica are unimportant. Tissue and blood helminths are nonexistent, intestinal helminthologic infection is mild, and malaria is not a problem. The population is predominantly of African make-up as is e.g. well demonstrated genetically in their haemoglobin types.

Our Cancer Registry in Jamaica could, we feel, be extended to include a section on kwashiorkor studies as was suggested above and we would be most happy to collaborate with any other study group.

c) To be really effective, we feel that such a study ought to be directed centrally, with perhaps a sub-organisation in 3 or 4 widely separated geographical

TABLE 6. *Dietary Surveys in Jamaica (Standard, 1958)*

Author	Date of Survey	Nature of Survey	Consumption/Head/Day					
			Calories	Protein (grams)	Animal sources %	Cereals %	Legumes %	Roots and other %
McCulloch	1942	Island balance sheet	1780	31	18	29	—	53
Platt	1944	Island balance sheet	2090	45	20	29	18	33
Dept. of Statistics	1953	Kingston: Family budgets	1700	48	52		48	
MacKay	1953	Rural children, 7–16 yr. Individual intakes	1690	49	33	50	10	7
Nutrition research team	1957–1958	Family questionnaire	1993	46	35	43	10	12

areas, say the Far East, India, Africa, Central and South America. A general plan set up by experienced workers, together with a medical statistician, must be outlined and each participating study group should be allocated a research fellow, whose specific duty would be the proper registration of data. These, apart from clinical observation, might conceivably include a list of possible aetiological factors common to the population at risk. The experience with our Cancer Registry indicates that a plan such as outlined above would have a good chance of yielding at least accurate figures for purposes of comparison.

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Panel Summary—Possibilities in Nutrition Research

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THESE THREE HOURS AROUND THE WORLD have demonstrated convincingly that numerous intriguing opportunities still exist for those seeking new fields of research in food and nutrition science. The panelists have given appropriate recognition to the classical deficiency diseases, both on a historical and on a current basis and have focused attention on a number of diverse environmental and dietary factors that may predispose to deficiency disease. Particularly, the panel has examined the occurrence of urinary calculi, especially bladder stones in young boys, the malabsorption syndrome, the neuromuscular abnormalities possibly related to dietary inadequacies, and the liver diseases, cirrhosis and cancer. In addition, the difficulties associated with the provision of vitamin-enriched staple foodstuffs in countries which have large malnourished groups in the population have been described.

The malabsorption syndrome is intriguing because of its varied nature and the severity of its effects. It is clearly important to know more definitely the roles of specific dietary abnormalities and of other possible factors, such as infection or genetic aberrations. The basic studies that have been described suggest the possibility that undue emphasis on steatorrhea has masked the importance of disturbed digestion of all three foodstuffs, protein, carbohydrate and fat, and especially that of protein. The question may well be asked if investigators generally have not assumed that our knowledge is more complete than is actually the case and if there is not a real need of defining more exactly the features of normal digestion and absorption which account for anabolism or catabolism according to the order and extent of appearance of the products of digestion in the blood stream.

The Bantu provides an exceedingly pertinent illustration of the complexity of the problems that determine the nutritional status of a population. One is impressed with the importance of obtaining accurate records associated with changes of nutritional status in such a group, not only records of foods eaten but also records of all other recognized factors including socio-economics, agriculture, public health and literacy. It is of the greatest importance to identify significant changes in the life of the Bantu, to oppose changes that are disadvantageous and avoidable, and to introduce compensatory measures for the offsetting of detrimental but inevitable changes.

The papers dealing with the relation of the diet to liver disease, neuromuscular disease and disease of the urinary tract suggest numerous possibilities and concepts which must be explored before it can be stated with certainty that the diseases in question are, or are not, of nutritional origin. The role of hereditary abnormalities cannot be minimized. Moreover, metabolism can be influenced by loss of enzyme activity for other reasons than by the absence of a gene for a specific enzyme. The chemical nature of an enzyme and of an enzymatic reaction requires indispensable amino acids for the protein component and, in most instances, one or other essential nutrients. Virtually this whole field is open for additional study to make certain of the role, if any, of nutrient imbalances, of toxins, of malabsorption, of genetic absence of enzymes and of single or multiple dietary deficiencies.

Nutritional knowledge must be applied and its application cannot be taken for granted. The development of motivation for nutritional health and the provision of the wherewithal for its attainment are often difficult to achieve. Nevertheless, nutritional science cannot evade responsibility for producing evidence of the relation of food to health and well being and for insuring that those in positions of authority are aware of the need of those measures which can be counted on to improve nutrition in a population.

The panel has encircled the world in less than three hours and has introduced and discussed in an excellent manner the possibilities in nutrition research. These include the new as well as new looks at the old. Subjects for study range from the phenomenon of the bladder stone in a boy in India to the complex causation of neuromuscular and liver disease in populations in many areas over the globe. Important also are the determination and interpretation of the relevancy for nutritional health of all of the many factors that are a part of the whole life of an individual or of a community. Not to be overlooked is the necessity of finding more efficient methods of extending nutrition education and of attaining optimum nutrition. In addition, a new and thorough look at some very old problems is urged, to the end that a rational understanding becomes possible, for example, for the still unexplained fact that beriberi rather than pellagra occurs in a man deprived of thiamine.

Symposium—World Food Needs and Food Resources

MORNING SESSION

Opening remarks

C. GLEN KING, Chairman
The Nutrition Foundation, New York City, USA

FOR THE FIRST TIME IN HISTORY, this generation has accomplished for a large part of the world a capacity to produce and distribute on a year-round basis enough food of good quality to meet the nutritional requirements for all segments of the population.

The goal of accomplishing this basic objective for all the world is now envisioned as a practical possibility, but we are far from the goal. One needs to travel in lands where hunger and deficiency diseases are still rampant, to appreciate the size and complexity of the task that lies ahead.

A great cloud in the form of a question mark on the horizon calls for our most thoughtful consideration, however. It is the inescapable need to anticipate, and meet objectively, the dual requirement of balancing population density with our capacity to provide food and other essentials for reasonable standards of living.

In the areas where greatest progress has been made in education and modern science, entire nations, large and small, are steadily progressing in their standards of living. At the same time, they are producing enough food of high nutritive value to be relatively secure from threats of hunger or specific nutrient deficiencies within the next 50 or 100 years. Their most damaging known form of malnutrition appears to be an excess of body fat. This problem is indeed a serious one and demands their greatest attention in nutrition research.

In contrast, roughly one-half of the world's population still contends with recurrent episodes of severe hunger. They face steadily a more tragic situation, however, in the prevaling high incidence of chronic deficiency diseases, among which a lack of good quality protein foods is generally the worst feature. The penalty is heaviest on infants, mothers, and small children. For them, the sickness and death rates are astoundingly high. Unless increased production of essential foods can more than offset the rising population made possible by control of infectious diseases in these areas, there is no prospect of building a stable or progressive social structure.

In the face of current trends and difficulties, the phrases 'Food for Peace' and 'Freedom from Hunger' are not mere slogans—they are expressions of great human need and great opportunities. The speakers on today's program will deal realistically with the issues that are at stake—some are full of promise, others are not so clear—but each p oblem is worthy of our best effort to study and find a-nswers

On behalf of the Congress, I want to thank each of the participants in the program for their respective contributions.

Welcome

EZRA TAFT BENSON, Secretary of Agriculture, USA

I FEEL GREATLY PRIVILEGED to have this opportunity to meet with so many eminent nutrition scientists from all over the world.

I hope all of you began the day with a good nutritious breakfast. Mrs. Benson made sure, as always, that I did. I had such a good breakfast, in fact, that I feel up to talking for at least an hour. But fortunately for you I also feel strong enough to resist that temptation.

During the past week in Washington you have been exchanging ideas on recent scientific advances in the field of food and nutrition, and you have been considering how this knowledge may be applied for human betterment. This is a most worthy endeavor. I congratulate you for what you are seeking to accomplish and wish you every possible success. You are a very select group of people—in the true sense a group of pioneers.

In these days when the world is awed by achievements in the conquest of space, there is a tendency to overlook the less glamorous, unspectacular work of people like yourselves.

The demanding tasks of studying the properties of food and encouraging its best use is not an easy one, and to all but those engaged in such work the rewards are far more difficult to recognize than are the results of seemingly more tangible accomplishments.

Yet you and I know—and the insurance companies' statisticians know—that the health and longevity of humanity is on a rapid upward spiral without parallel in all the long centuries of history before the 20th Century. In this development better nutrition is no doubt playing a highly important part.

I want to take this opportunity to commend our own nutritionists and home economics specialists for what they have done in helping bring about a great improvement in the diets of our people here in the United States In the Benson family we have deep respect for that branch of knowledge known as home economics. Three of our four girls, who are or have been in college, selected "home ec." as their major subject—and our fourth daughter plans to do the same.

During the past half century we have seen a revolutionary advance in scientific knowledge about foods and nutritional needs. But our knowledge is still far from complete. We look to you—and to the science of the future—to give us new insight into the relation of food to health so that we may employ our food resources even more wisely.

We must learn more about how much of different kinds of food people need to maintain excellent health—and I mean people of every age and in every occupation. We must learn more about the over-all economy and nutritive values of different foods. We must learn more about how they supplement one another for normal nutrition. We need to know how much of various items of food different population groups customarily use, and the trends in each case.

The key to all this is research—nutrition research, food research, agricultural research.

Such research is a very broad endeavor. It must be carried out on a very broad front, involving both public and private components. Here in the United States, for example, private expenditures for food and agricultural research are larger than all public expenditures for this purpose. But all this research, both public and private, is in a broad sense but one single force. Our U.S. Department of Agriculture research is conducted with the fullest possible awareness of the related programs of all other public and private agencies. In many cases, the research is cooperative.

Agriculture's capacity to produce depends on many factors. But with every passing day, research and education become more important. Here and throughout the world, mankind needs greater technical knowledge about soils, water, fertilizers, disease and insect control, agricultural engineering, and cultural and management practices for crops and livestock. Research, more and more and still more research, is the best insurance that the world will not only go on meeting its future food requirements but will be able steadily to raise its levels of nutrition.

But it is not enough simply to increase food supplies. Foods must be effectively processed and distributed. They must be available at the times and places needed by the people who use them—available at prices people can afford to pay. And consumers, too, must be informed and motivated so that they will select and use foods that will support good nutrition. All of this calls for research—and for educational programs to carry the results of research to the people who can use them.

You have seen no doubt the 1959 Yearbook of Agriculture, *Food*. This, I believe, is proving to be the most popular of all the USDA Yearbooks. Copies printed or in process of being printed now exceed 400,000. It is a prime example of what I consider good research and good education combined. This book was developed to meet the express needs of nutrition leaders in this country for authoritative and up-to-date reference information on foods and nutrition. It is being read by

parents and teachers, by business people and farmers, and by consumers generally. As part of its contribution to this conference, the U.S. Department of Agriculture was pleased that it could make available to each member a copy of this Yearbook. We hope that it will be a useful reference in developing strong nutrition programs in your respective countries.

In today's symposium, "World Food Needs and Food Resources," persons with specialized knowledge will discuss in detail some of the important aspects of this problem of food production and consumption. We know that much has already been accomplished by governments and intergovernmental agencies. Privately financed foundations, too, have made substantial contributions to the improvement of agriculture and levels of nutrition in many countries.

Without going into detail, let me take just a moment to sketch for you some outlines of the Food for Peace Program in which several governments and many private agencies are participating and which no doubt the President alluded to in his opening remarks before your conference.

The Food for Peace Program was inaugurated last year at the suggestion of President Eisenhower. This is not so much a new program as a new emphasis. The food relief operations administered by President Herbert Hoover after World War I were a food for peace program. The post World War II food relief program also was a food for peace operation.

With the Agricultural Trade Development and Assistance Act of 1954, known as Public Law 480, we entered a new phase.

The present Food for Peace Program is a continuation and an intensification of these activities.

Twenty voluntary agencies and the United Nations Children's Fund are currently making donations of nonfat dry milk, flour, cornmeal, and rice to more than 60 million persons in over 90 countries and territories around the world. Since 1953 nearly 9 billion pounds of U.S. surplus foods (70 percent dairy products) costing about $1½ billion have been donated and distributed overseas.

This is truly a stupendous accomplishment. There has been nothing quite like it in all the previous history of the world.

In the past seven fiscal years over $8½ billion worth of U.S. surplus farm products has been moved overseas under various special government programs. This was in addition to commercial agricultural exports with a market value of about $18 billion, sold for dollars. All of these exports—a record $26½ billion in seven years—helped the cause of peace. Yes, dollar markets, too, are food and fiber for peace in that high quality products from our farms serve the well-being and improve the standard of living of people in all lands.

Another phase of Food for Peace involves helping needy foreign countries produce more of their own food supplies. More than a thousand U.S. agricultural technicians are working in many of the newly developing countries bringing American farming and processing know-how to foreign producers. And large amounts of foreign currencies generated by Public Law 480 are being loaned or granted to foreign governments for economic development, including agricultural improvement.

Food for Peace is cooperative, too, with other free world nations that are rendering vital aid to people outside their borders. This aid is extended in part on a country-to-country basis. Some is provided through and in consultations with international organizations, such as the Wheat Utilization Committee. On this Committee are represented the major wheat-exporting countries—Argentina, Australia, Canada, France, and the United States. With the FAO as an adviser-observer, the Wheat Utilization Committee is investigating the possibility of increasing and making more effective coordinated use of wheat surpluses to promote economic development, improve nutritional standards, and expand world commercial trade in wheat.

If we are to reach our goal of adequate levels of nutrition for peoples throughout the world, nations must continue to acquire and exchange information on technical and economic matters. They must agree on problems of trade. They must work together to solve the problems of adequate and efficient production and distribution of foods.

I know—because I have seen with my own eyes—that people all over the world desire two things above all. They desire peace and they desire decent standards of living. I've been impressed that people all over the world are very much the same. They desire to raise families, raise standards of living, and to do good.

In 1946, I visited 13 countries of war-torn Europe and I lived among the people of Europe for nearly a year. I saw some nations flat on their backs economically. I saw people who were hungry and cold—some near starvation. It's a terrible thing to look at people who are starving and to know you can't do anything about it. I was with them when they gathered in unheated, unlighted, bombed-out buildings. I saw thousands of refugees, who had been driven from their once happy homes to destinations unknown. I visited some of their new living quarters—their shacks—where as many as 22 persons—four complete families—were living in one room.

At that time it seemed to me that there could never be quite enough food to meet even the minimum requirements of a hungry world. The problem was poverty, scarcity, hunger.

About three weeks ago I returned from a trade and market development trip to Western Europe and the Middle East. This is one of several such trips we have made to every continent in the world in the past five years. What tremendous progress has been made in world food production in recent years! Despite a sharp increase in population, per capita production and consumption of food are higher now than they were before the war.

Yes, this is truly immense progress—yet it would be idle to say that we have advanced on as broad a front as we need to.

Though world agricultural production has exceeded prewar levels, per capita output in some important areas, such as parts of the Far East and Latin America, is still below prewar.

It remains for us then to push forward with new vigor in the interests of peace and plenty.

From all my travels—whether here in the United States or abroad—one impression stands out: The dedication of farmers all over the world to their job of helping to feed and clothe their fellowmen. Their efforts, of course, must also be aimed at obtaining a better living for themselves and their families. But there is a special dedication of purpose among farm people that seems to set them apart. It stems, no doubt, from working creatively with living things. Farmers are producers. They are men of the soil. They have the opportunity to be, in a special way, men of God. Their common purpose is to produce—to work in partnership with the Creator in producing bountiful harvests. And this is a fact of great meaning—of great hope—in the present era.

The world's people are awakening to the hope that their long-awaited basic wants and needs *can* be satisfied—and their wants range far beyond food, shelter, and clothing.

An elder statesman of the Orient once put it this way: "We want rice and respect—but we want respect first."

I believe it is to the glory of the world's nutritionists that your aim has been to make possible for the world's people both more rice and respect—and, I believe, more respect first.

In welcoming you to this symposium I give you these words of our great President, Dwight D. Eisenhower:

"The peace we seek, founded upon decent trust and cooperative effort among nations, can be fortified, not by weapons of war but by wheat and by cotton, by milk and by wool, by meat and by timber and by rice . . .

"We are ready . . . to dedicate our strength to serving the *needs*, rather than the *fears*, of the world."

So my good friends, delegates to this great Congress, God speed us on our way toward this worthy objective.

The world's increasing population

JAMES BONNER

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THE MOST BASIC FACT about the earth's human population is that it is increasing very rapidly. We are all, I am sure, acquainted with the numerology of the matter —that each second the population of the earth increases by one, each day by some 100,000, and each year by some 45,000,000 new net individuals. The yearly increase in the earth's population is today greater than the total population of the world at the dawn of agriculture, some 8,000 years ago. We all know that populations unchecked grow exponentially. They double every so many years. Our human numbers are growing at a rate which causes the total to double every 40 years or so— 2.7 billion today, 2 × 2.7 billion by the year 2000, 4 × 2.7 by the year 2040, and so on. Well, now, the next fact about population, and on which I think we can all agree, is that such expansion cannot and will not go on indefinitely. I am sure that we are all acquainted with the calculation that at the present rate of population growth the land area of the earth will in 700 years be covered solidly with people standing shoulder to shoulder and without room to sit down. This is in itself an unpleasant enough prospect, but I have made a further calculation with an even more distasteful conclusion concerning what will happen if human numbers continue to grow unchecked after the 700-year mark. People will have, of course, to be stacked on top of one another in tiers; a second tier of solid people, a third tier of solid people, a fourth, and so on. More and more rapidly, people will be added to the surface until 7000 years from today the earth's surface will be expanding out into space with the speed of light. This would, if it were only physically feasible, solve the earth's population problem—a space sputnik of people speeding out to the edges of the universe. But this is all of course impossible. The exercise merely serves to stress again that the rapid increase in human numbers which the world now experiences cannot last for long. In the long perspective of history it will be at most a transient perturbation, lasting but a second in time.

That the growth of the human population will ultimately cease is small cause for comfort today. Rapid population growth is already robbing the peoples of many lands of the full benefits of industrial and agricultural development. Rapid population growth has and will lead to poverty, hunger, and misery for millions of human beings. If we were lemmings or grasshoppers or rabbits, there would be nothing we could do about the situation. Since we are not lemmings, but intelligent human beings, we can do something about it. We can try to understand the factors which determine rate of increase of people and we can try to decide to the best of our ability what is an appropriate population size and rate of increase, and we can try to spread this knowledge and cause it to be acted upon.

Let us start our analysis with the history of population growth. I suppose that we are all aware that human numbers have increased but slowly during most of the 100,000 years since man came into being. Eight thousand years ago, at the threshold of agriculture, our species numbered perhaps 10 million in total. This increased to perhaps 200 million people by the beginning of the Christian era. Over this long period, human numbers doubled less frequently than once per 1,000 years. The pattern of slow population increase, increase at rates of less than 0.1 % per year, continued indeed up until 300 years ago. In the early 16 hundreds the time-honored pattern of population growth was abruptly supplanted by our present pattern—a pattern in which the population increases more and more rapidly, in which the rate of increase itself increases. World population doubled in the two centuries between 1600 and 1800; doubled again in the 100 years between 1800 and 1900; and now is expanding at a rate which promises a doubling every 40 years or thereabouts.

The reasons for this vast surge in human numbers, beginning in the 1600's, are not far to seek. It is quite clear that for most of man's time on earth, some 4 or 5 thousand generations, his numbers have been limited by famine and disease. Famine we have slowly alleviated by slowly improving methods of producing food by agriculture and of carrying food from regions of sufficiency to regions of deficiency. Disease we have slowly brought under control by improving practices of public health. It is the rise of modern science and technology which has brought into being this ever increasing horde of human beings. The factors which have caused human numbers to increase are ones which decrease death rate and increase life's span. Birth rate on the contrary has probably remained constant or nearly so for most of our human population over the past 10,000 years or perhaps for the entire 100,000 years. Only for a small portion of the human species and only in recent times

have birth rates decreased below the primitive maximum set by the ultimate human fertility. This exception is the one that has to do with the march of industrialization in those lands which have felt its full impact.

Industrial development, development of the technical industrial culture, commenced in Britain in the 18th century, spread to western Europe, jumped the Atlantic to the New World, the Pacific to Japan, and has emerged most recently in the Soviet Union. In each of these regions the course of industrialization has been attended by a characteristic pattern of changing birth and death rates—a pattern of transition from the high birth rate-high death rate condition of primitive agrarian culture, to the low birth rate-low death rate state of the highly developed industrial culture. It has been characteristic of this transition that with the growth of industrial development, death rates drop first, birth rates remaining high. With a lag of perhaps a generation, birth rates start also to decline. And in the highly industrialized country, we find both death and birth rates stabilized at new low values. To accomplish this transition has required 90 years in Japan—the country in which it has most recently been consummated—almost four generations during which birth rates greatly exceeded death rates and during which the population of Japan has expanded by over three-fold.

Much of our world stands today in the midst of the transition from the high birth rate-high death rate agrarian culture to the low birth rate-low death rate scientific-technological industrial culture. If population trends of the future follow those of the past, we can hope that industrialization, as it spreads and develops throughout the world, will bring in its wake a slowing of population growth. We can forecast future human numbers on the assumption that demographic trends will follow those, say, of Japan. On this basis we must conclude that the world's population cannot approach stabilization before another 90 years and that by this time will have reached some three times its present level, attaining some 8 billion souls.

Population projections for the long-range future have in fact been made primarily on the basis which we have just discussed, on the basis that future demographic trends will follow past performance. This is the most straightforward way to make such projections. We already know, however, that this projection is wrong and underestimates the rate of future growth of population. It is wrong because the technology of control of death rate can now be spread much more rapidly than in the past. The slowly sinking death rate of England or of Japan during the course of industrialization is replaced today by a rapidly plunging death rate in the newly industrializing countries. The death rate has been, for example, cut in half in Ceylon in 7 years—something which took 70 years in Britain and 40 years in Japan. It is feasible to cut malarial deaths dramatically in one year by the simple expedient of the widespread use of insecticides, and so on. We have dramatically improved the technology of control of death rate as well as the technology of dissemination of this technology.

And this is good. Everybody is for minimization of human misery, and abolition of unnecessary untimely death is one step in this direction. But our conscious technology does not yet extend to control of birth rate. The course of industrial development, as we see it today, is accompanied by dramatic decreases in death rate and by fertility which remains undiminished for at least a generation. These are the circumstances under which we see populations expanding at an explosive rate, growing as they are in some areas at the rate of over 3% per annum, doubling population in a single generation. Suppose then that death rates drop rapidly in the developing areas of the world but that birth rate trends follow those of the past. On this basis we must expect not a tripling but a quintupling or more of the world population before near population stabilization can be achieved, 90 or 100 years from today. On this basis we must expect 100 years from now a world population of more like 5 or 6 times today's population, some 15 billion people.

But of course these projections too are suspect. They are open to error. We may recognize the possibility that just as conscious and effective control of death rate has spread through the world at an ever-increasing rate, conscious and effective planning and control of birth rate may also spread at a rate more rapid than has been the case in the past. And I believe that there is real reason to suspect that this may happen. New forces are at work.

In the first place, economists are much clearer today than ever before in their understanding and formulation of the influences of rate of population growth on industrialization and rate of increase of individual living standards. I cite as one example the projections of Coale and Hoover relating to the changing status of a developing country over a 30-year period. We start with a birth rate of 40 per 1000 per year and a rapidly dropping death rate. We plough 8% of the gross national income into development each year—into factories, transport, schools, agriculture. This is a reasonable model to start with and approximates that actually found in India today. Suppose now that the birth rate remains constant at 40 per 1000 per year for the whole 30-year period. The population doubles, and in spite of the substantial increase in gross national income, the lot of each individual is actually worse at the end than at the beginning of the period.

Let us as an alternative imagine that we start with the same initial birth rate but that this drops steadily to 20 per 1000 per year, one-half its initial value, in 30 years. We do this within the same framework of death rates and rate of investment. During the 30-year period, population increases by 50%, to be sure, but nonetheless the real income of each individual is, at the end of the 30-year period, 3½ times what it was at the beginning and 4 times that of the unhappy individuals who live

in the model of constant high birth rate. This is economics, to be sure, but it isn't very complicated. The punch line is that the lot of our children can be bettered very rapidly if we have fewer of them. Population control is one sure way to speed the course of development and to increase the health and dignity of a people. This message can be understood at once by some individuals in each and every country—by, for example, responsible officials. And the tools for making this message felt and understood by whole populations are at hand today as they have never been before: radio, television, the advertising art, some understanding of the wellsprings of human motivation. I conclude, therefore, that other countries may well follow the path already indicated in outline by Japan and embark upon deliberate population control programs on the basis not only of national welfare but on the basis of an appeal to each citizen to maximize his own and his children's personal welfare.

There has been in the recent past much discussion of and attention to programs for the increase of agricultural productivity in those regions of the world in which people are undernourished. Such programs have in fact been undertaken with energy and imagination and on a very large scale in many countries. And indeed the greatest vigor and attention to increase of food production is required in the presently emerging industrial regions just to keep up with increasing population. Great hopes have been held out for improving the lot of hungry peoples, but it seems clear that the lot of the individual hungry person can scarcely be improved even by the most vigorous agricultural development program as long as high rates of population increase continue in the same region. We know a great deal about the technology of increasing agricultural production in underdeveloped areas, and one of the things that we know is that it's a difficult, a slow and a long job. Let me cite the experience in this matter of the Republic of Mexico, a country which over a long period has increased its agricultural productivity more rapidly than any other in modern times. Thus over a period of 20 years, agricultural production has been increased by over 60%, increased at the rate of 3% a year for over 20 years. This achievement has been the result of the intense application of every kind of intellectual and fiscal resource, What has been achieved in Mexico can in principle be achieved elsewhere but it is already clear that the pace is too rapid, the fiscal strain too great for many regions. It is more reasonable to suppose that agricultural productivity can generally be raised over long periods at a rate of something like 2% a year. And while increasing food production at the rate of 2% a year will indeed double food production in half a century, it cannot double the amount of food available per person as long as population increases at the rate of 2% or more per year. And I feel therefore that this matter should be clearly understood and looked in the face by responsible authorities and by each one of us. There appears to be no real hope that the lot of each individual in a hungry country can be increased and bettered even by the most vigorous agricultural development program until rate of population increase is slowed.

So much, then, for the effects of high rate of population increase upon individual nutrition and individual standard of living. Let us now talk about population in another way. Let us talk about the ultimate limits, about what is a reasonable human population for the world. What population level would be consistent with physical principles and with a reasonable standard of living of each individual? The physical principles which govern the matter appear to me to be those which tell us how much food can be produced each year. Human food is the factor which sets a physical limit on human population, and in turn there are physical principles which set an upper limit on the food producible per unit area per unit time.

The most generous estimate of people supportable by our earth is roughly 50×10^9 people. This estimate has been made both by Meier and by myself. To support this number would require a very impressive economy indeed—an economy which is impressive even if we look only at that facet which is devoted to food production. It would require the cultivation of all potentially cultivatable land; it would require the irrigation with reclaimed sea water of the earth's deserts, and so on. It would be a highly vulnerable culture in which any major catastrophe, such as breakdown of the industrial power network, would result in starvation of 9 out of 10 of the population. It would be a crowded world—20 times as crowded as today. It would be a world in which all animal protoplasm would be replaced by human protoplasm. One could not afford the luxury of maintaining pigs or cows or horses or dogs or cats, or even, probably, strange creatures in zoos. All edible material would have to go down the throats of hungry people.

A less intensive food economy—one approximating that of Japan today—would suffice to support about 16×10^9 people. In such a world, the present Japanese level of agricultural productivity would have to spread over not only all of the world's presently cultivated acres but also over an additional area which would increase the total by 50%. The world's peoples would have to all agree to a vegetarian diet and to deny themselves the luxury of animal products with their inherent caloric inefficiency. This is clearly an intensive economy even though it is much less intensive than my first model.

Finally, a population of some 8 to 10 billion people should be supportable on the earth's surface under a food production program characterized by the efficiency of present-day Japan, spread over all of Asia, and by the efficiency of present-day western Europe, spread over all of the rest of the earth's agricultural acres which have in the meantime been expanded by 50%. This level of agricultural productivity should in principle be attainable within another 75 to 100 years at the rate of 2% increase per year.

What then would be an optimum population of human

beings on our planet? Quite evidently an optimum population would be one not too close to the limit, for then catastrophe might wipe out our species. Such catastrophe might consist in unpredicted fluctuations in food production or in other resource availability. In addition, since we may never be able to control our population precisely, we might sometime overshoot and reduce billions of human beings to poverty. It would be nice, too, to have our population sufficiently far from the limit so that we might afford the luxury of having other creatures about us—a few cats and dogs perhaps, and in fact a going population of each of the wild creatures with which Nature has supplied us for company.

These arguments and considerations, then, put forcefully the case for limiting human population by some means other than the premature killing off of living people. Can this be done? In actual fact, what we ordinarily refer to as the fully stabilized countries are not characterized by really stabilized populations. Their populations expand, rather, at a rate which is uneven in time and in segments of population. By and large, the so-called stabilized populations of western Europe are growing at the rate of ½% or so per year. This rate, although it is compatible with a high rate of increase of individual income, cannot persist forever. Even at the rate of ½% increase per year, human populations double in 140 years. It will be necessary in the long run to find better methods of wholly controlling and stabilizing human numbers. Among the suggestions that have been made along this line is the question of what might be done to introduce new cultural patterns in which great prestige is given to positions in which the individual cannot procreate. Meier, for example, proposes the creation of satisfying lifetime roles which do not require and are not compatible with parenthood, and suggests all professions which require much travel for this role.

But the exact method of final population stabilization is less important today than is recognition of the need for and indeed the inevitability of such stabilization. Population stabilization is the ultimate consequence of any morality which places weight on the well being, productivity, and dignity of each individual human being.

Prospective world production and distribution of food

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AT NO TIME IN HISTORY have all people everywhere been adequately nourished. Yet the world has the soil, water, and sunshine to produce several times the amount of food that is being produced today.

We recognize, however, that it takes more than these physical resources alone to provide nutritious diets for everyone. Putting food on the world's dinner table requires the efforts of hundreds of millions of people, concerned not only with production but also with processing, distribution, and consumption of food.

The job deeply involves science and technology, applied on a worldwide scale. This in itself is difficult in the extreme. Even so, the technological problems may be easier to overcome than the educational, economic, and social or institutional barriers that stand in the way of developing an efficient agriculture throughout the world.

Since these latter problems will be discussed by the next speaker, I shall limit my remarks to the technical feasibility of increasing the world food supply—without trying to predict whether the increases will, in fact, take place.

In seeking higher levels of food production, there are two obvious courses that can be followed. One is to increase yields on existing cropland. The other is to bring new lands into cultivation.

Progress in several areas of the world has demonstrated that improved farming techniques can increase production enormously. In Europe, before the rise of agricultural technology, crop yields remained virtually unchanged century after century. Grain production, for example, remained under 10 bushels per acre from the time of the Roman Empire until just before the French Revolution. Then, with the development and adoption of better farming methods, production started to climb. Per-acre yields of many crops have more than tripled since 1800. In fact, with presently known scientific facts, farmers in most of northwestern Europe are now approaching the maximum in economically attainable yields in relation to soil resources.

In the United States, food production was increased for 300 years mainly by bringing new lands into cultivation. Significant increases in average yields per acre were not obtained until after 1940. In order to meet the needs of World War II, farmers began applying on a large scale the results of research that had been accumulating for 50 years. Yields of most crops have been increasing since that time, and prospects are good for still further progress in the years ahead.

By taking into account yield increases that have been attained in the various soil and climatic areas of the United States, it is possible to assess with a reasonable degree of accuracy the yield potentials in other countries where similar physical conditions exist and where present yields are low.

About 15 years ago, Department scientists used this means to project yields per acre and total world food production attainable by 1960. The data included estimated yield increases attainable for several important crops in the Soviet Union, India, and China—three of the world's most populous countries.

Using 1935–39 as a base period, the scientists estimated, for example, that by 1960 the Soviet Union could increase per-acre yields of wheat 20%, corn 23%, and potatoes 48%. In India, attainable increases included 53% for rice, 87% for wheat, and 55% for corn. It was estimated that China could increase yields of rice 33%, wheat 21, corn 45, and potatoes 50%. The percentage increases in yields considered attainable in these countries by 1960 were substantially equivalent to those considered attainable in the United States by 1950. They represented a total increase of about one-third over average production during the period 1935–39.

Now that we have reached the year 1960, let us see what has happened to yields in the United States. The fact is that farmers in this country, even before 1960, exceeded by a wide margin the yields predicted 15 years ago.

For example, in 1945 the scientists estimated that by 1960 the yield of rice could be increased 13%; the increase has actually been 58% (1957–59 average). The projected increase for wheat was 18%; the actual increase is 90%. For corn, the figures are 31% estimated, and 79% actual. And for potatoes, the yield increase considered attainable by 1960 was 23%, whereas the actual yield increase has already reached 137%.

In considering production potentials in the United States, the scientists merely assumed a general accep-

tance of the technology then being used by millions of farmers. They did not take into account the greatly accelerated rate at which both existing and new technology would be applied.

During these 15 years, the use of fertilizer has more than doubled. It is of better quality and is being applied with ever greater precision and effectiveness. Irrigation has been extended to humid areas to supplement rainfall during critical growing periods. Increasingly effective chemical and biological techniques have been devised for controlling insects, diseases, and weeds. New plant germ plasm from many parts of the world has been incorporated into our commercial crop varieties, making them more responsive to fertilizer, more resistant to diseases and other pests, and better adapted to soil, water, and climatic conditions. Improvements in livestock and poultry breeding, feeding, and management have made it possible for farmers to obtain 13% more pork per sow, 34% more milk per cow, and 36% more eggs per hen.

As a result of these and other applications of acience and technology in the United States, fewer farmers, using about the same total acreage, are now producing about 60% more farm commodities than they produced during the period 1935–39. This compares with an increase of about 25% considered attainable 15 years ago.

The fact that wide variations in levels of production still exist in different areas of the world gives us reason to be optimistic about yield improvements in those areas where farm output is now low. For example, in 1957 the yield of wheat in Denmark was nearly four times the world average, six times that of India, more than eight times that of Africa, and twice the yield of wheat in the United States. The rice yield in the Far East, which equalled the 1957 world average and was nearly double the yield in Africa, nevertheless amounted to only half the average yield of rice in North America.

Certainly it is safe to say, in light of these figures, and considering the technical knowledge available today, that the potentials for increasing production on existing croplands are significantly greater than they were 15 years ago.

In fact, if we consider technical feasibility alone, it would be possible to meet and exceed present world food needs without adding any new cropland. Estimates made 15 years ago on total attainable production from existing cropland indicated that world food needs in 1960 could be more than met for roots, tubers, and sugar, and virtually met for cereals, fats, and oils. Attainable production of dry beans and peas, nuts, fruits and vegetables, and milk were estimated as likely to be short of 1960 needs.

We all know, of course, that in most parts of the world the production considered in 1945 as attainable by 1960 did not take place. But here again, the greater-than-anticipated yields of commodities in the United States, plus the recent gains in Mexico, Japan, Turkey, the Philippines, and several other countries, indicate what could be done under reasonably favorable conditions.

If we consider new lands that could be brought into production, the possibilities are even greater.

Only about 10% of the total land area of the world is now in cultivation. Some 60% is in areas of perpetual snow and ice, tundra, mountains, and deserts. We can assume, for the present at least, that these areas have no practical possibilities for expansion of agriculture. This leaves about 30% of the world's lands—three times the amount now cultivated—that are not now in agriculture but which have soil, water, and climatic conditions that theoretically permit cultivation.

These lands are found mainly among the podzol soil groups in the North Temperate Zone and the red soils of the tropics and subtropics. For each additional 10% of the northern soils that could be brought into cultivation, we would have 300 million new acres for food production. And for each 10% of the tropical and subtropical soils that could be brought into cultivation, about 450 million acres would be added.

I have stated that it is technically feasible to meet current world food needs with lands currently cultivated. I have also stated that additional land totaling three times the acreage now in cultivation is potentially available for use. We thus have resources to feed a much larger population than now exists on the earth.

Considering, however, the rate at which the world's population is increasing, it is entirely possible that all available land will eventually be needed to provide enough food for all the people.

The primary question, then, is: How can we attain the production potential of the world's available soil resources—both those now in use and those yet to be developed?

Up to now I have been talking largely in terms of averages—for countries, for soil groups, and for the world. We all realize, however, that food is produced on hundreds of millions of individual farms, each one different in some way or other. No farm family tills an 'average' soil. Soils vary the whole way in productivity. Some are good to begin with, others can be built up, and still others may never reach high production.

Some soils are adapted to a narrow range of crops with relatively low yields—for example, the semi-arid grasslands, on which a large part of the world's bread grains are produced. In those areas, farms are large and far apart, and only a small portion of the family's food comes directly from the soil. But some of the soils in the humid tropics are adapted to a very wide range of crops under intensive culture with high yields. Here, farms may be small and close together, and a large part of the family's food is grown directly on the soil.

Institutional needs—such as fertilizers, seeds, pesticides, credit, insurance, and marketing facilities—must be supplied, but they vary markedly with the different kinds of farm enterprises and the educational, social, and economic conditions that prevail locally.

Farmers usually are conservative from necessity. Very few of them can risk their family's welfare on an uncertain innovation. In many parts of the world, therefore, the farmer tends to follow the practices of his father and grandfather. This basis for decision must be changed, if he is to benefit from science and technology. To his experiences and tradition must be added knowledge and methods developed by other people and adapted to his own farm—whether it be a small mountain dairy farm, a tropical banana plantation, a grain farm on the plains, or a sheep ranch in the semi-desert.

To take advantage of science and technology, most farming systems must be planned ahead for a period of years. The soils need to be improved, to be built up through combinations of such practices as liming, fertilizing, establishing grass meadows, planting shelter belts, building stock ponds, using long rotations with grass or shade crops, and putting in devices for drainage and irrigation and for controlling run-off and erosion. Although these improvements may not result in substantial increases in production and net income the first few years, the eventual benefits will far outweigh the time and labor invested.

Some practices, of course, can bring immediate returns. On many farms, especially in underdeveloped areas, a profitable start can be made with new seeds of superior varieties of familiar crops, simple treatments to control diseases like smut of wheat, fertilizers and improved organic compost, better storage bins, and other simple improvements. The confidence engendered by beneficial results obtained from these innovations will lay the groundwork for better tools and machines, disease control among animals, new crops, new pest-control methods, and the more effective combinations of these and other improvements.

The importance of combining several practices for greater efficiency cannot be overstressed. Until recently, even in the more advanced countries, most agricultural techniques have been developed and recommended singly. Farmers are given recommendations on the housing of pigs, feeding of chickens, fertilization of corn, selection of wheat varieties, irrigation of citrus, construction of land terraces, and hundreds of other important farm operations. But, in most cases, the recommendations are based on the assumption that somehow the individual farmer can take any one of these separate practices and apply it with good results.

Both research and farm experience, however, are showing that far greater benefits are obtained when two or more improved practices are applied simultaneously. In an experiment with corn in Punjab, India, for example, a local variety that produced 25 bushels per acre, when grown in the traditional manner, produced 28 bushels when fertilizer and water control were added. An improved variety grown in the traditional manner also produced 28 bushels per acre. But when this improved variety was given the proper amount of fertilizer and water, the yield of corn increased to around 100 bushels. Here in the United States, average corn yields in North Carolina have been increased 50 to 75% by combining high-yielding hybrids, greater plant population, and improved fertilization and soil management.

The interactions among soil, water, plants, and cultural practices are especially important in making international exchanges of technology. Full account must be taken of the conditions existing both in the country where the technology was developed and in the country to which it is transferred.

A good recommendation for the use of lime in one country, for example, may be ruinous in another country with different soils and different crops. Many of the modern crop varieties in the United States are tailored to give high response to fertilizer and proper timing and application of water. They may be resistant to diseases that occur here but highly susceptible to those that occur somewhere else. Before these varieties are recommended in other countries, they should be tried out within the area where they will be grown, to determine how they respond under local soil, water, and other environmental conditions. Actually, improved crop varieties offer most when they are used in breeding new varieties especially fitted to the complex elements of the local environment.

Then, too, the use of one technique often depends upon the availability of other techniques. For example, many modern farm operations in the United States and western Europe depend upon electric power, which is not yet available in many other places. Also, technical skills of farmers in a given area may not be adequate for a practice that is otherwise suited to them. Skill in handling machinery is a case in point. In Europe and the United States farmers went from hand tools to animal-drawn equipment and finally to tractors. In many undeveloped areas the intermediate step, which would require new work animals, is not practicable. And in these areas, farmers must go from improved hand tools to simple tractors and tractor-drawn machines with as few adjustments as possible.

The point I want to stress is that if farmers, especially those in underdeveloped countries, are to progress in an orderly and effective manner, they must have advisory services that are based on knowledge of scientific principles combined with knowledge of local conditions. They cannot depend solely on techniques that have proved successful in other places where conditions may be entirely different.

Although much of the agricultural progress in the United States and Europe is the result of an efficient extension or advisory service, we must remember that from the beginning this service has been an inseparable part of agricultural research. Many technicians in the more advanced countries take for granted the body of scientific knowledge that has made possible the technology they use.

It is this basic scientific knowledge that can most

easily be transferred. The laws governing the chemistry of soils, for example, are the same in the United States, India, Argentina, Rhodesia, and everywhere else. But their application varies widely with the different kinds of soil and the crops grown in each country.

With sound training in the basic sciences, technicians can analyze local problems and develop efficient methods for local use based on their knowledge of the basic principles involved in soil and crop management. Lacking knowledge of basic principles and of the scientific method, they are not likely to be very successful when working in an unfamiliar environment. This is as true of the United States technician who does advisory work in India, as it is of the Indian student who is trained in the methods used in the United States and returns to do advisory work in his own country.

Even when basic principles are transferred from other countries, there must be scientists available with the competence to understand these principles and to interpret them for technicians who work directly with local farmers. In soil science, for example, many basic principles may be transferred from temperate regions to tropical regions that are valuable for the work at a research institute, but only a few that are directly appropriate to an extension program.

This means, then, that advisory programs for sustained high production depend upon effective research programs carried on within each country. Full use must be made of all the sciences relevant to agriculture, and there should be a proper balance between research to discover new scientific principles and research to develop practical methods and techniques for use on farms.

I want to stress that much good research is being done in many countries. We are apt to be most familiar with the work in northwestern Europe, Canada, Australia, and New Zealand. But South Africa has long been famous for research in the veterinary field and also has one of the world's leading citrus stations. Japan has excellent facilities for research on rice breeding and production. The tiny island of Fiji has three agricultural research stations. These and other research centers are making important contributions, but the fact remains that in most of the underdeveloped areas of the world, agricultural research facilities and personnel are sadly lacking. Perhaps even more serious is the relatively low esteem in which agricultural scientists are held in many countries. I believe that this unfortunate situation must change—that a well-rounded research program, supported alike by government and citizens, is a vital part of any nation's effort to increase production of food.

In fact, I am convinced that in research lies our greatest hope for eliminating hunger throughout the world. In my opinion, we are only now entering the golden era of biological science. It will unfold as we gain understanding of the cell as the unit of life. Such understanding will be particularly important to agriculture. If we can better understand and control the mechanisms and functions of living cells, we will have vastly increased ability to breed more productive, higher quality crops and livestock; to control or eradicate diseases and insect pests; to maintain the quality of farm products during processing and marketing; to find new uses for farm-grown raw materials; and to improve human nutrition.

If we could unravel some of the chemical processes occurring within living cells, we would be much nearer to an understanding of the fundamental nature of life. What is the nature of the organization within viruses which makes it possible for them to reproduce themselves? What is the nature of the chemical or physical changes that result in virus mutations? If we had the answers to these questions, we might be able to produce mild strains of many viruses capable of immunizing plants and animals against diseases caused by more virulent strains. Or we might develop chemical methods for destroying the viruses.

A part of the riddle is that all proteins are composed of the same amino acids combined in various ways and proportions. Yet one protein turns out to be inert, another with nucleic acid is a virus, and still another is an enzyme. And enzymes are the controlling catalysts in metabolic processes.

There are many different enzymes—at least one for each process. Yet there is an underlying unity in the molecules that nature uses for its processes. Similar enzyme molecules catalyse similar reactions both in one-cell organisms and in higher animals. This gives us hope that in our studies of plants and animals and microorganisms we will one day be able to fit the pieces of our jig-saw puzzle together into an understanding of the nature and behavior of enzymes and viruses, and, in fact, of all proteins.

This knowledge will help us to understand metabolic processes in cells. It could well turn out that major errors in cell metabolism may be involved in cancer, and that the accumulative effect of minor metabolic errors or imbalances may contribute to the aging process. If we can learn more about cell metabolism and the cause or control of errors in this basic life process, it could benefit not only agriculture but all mankind.

Recent evidence indicates that the gene may not be the fundamental unit of heredity, in terms of composition, but may consist of chemical subunits. An understanding of the chemical organization of these fundamental units, how they reproduce themselves, and how they produce their effects in the organism would provide valuable new methods of genetic control in plant and animal populations. If we can understand the chemistry of genes, we may learn how to modify them by chemical treatment. From this it follows that metabolic processes would become subject to modification.

The implications of such mastery over biological behavior are tremendous. If specific desirable changes could be made, the rate and amount of genetic improvement in animal and plant populations would far exceed anything now possible.

I have discussed some of the ways in which science

and technology can be used to increase the production of food. But this food must also be distributed to millions of people living in towns and cities. Although I have not discussed specifically the techniques involved in improving food distribution, certainly the improvements required are as important and perhaps more difficult to accomplish than those involved in increasing food production.

In the first place, until distribution channels are available, farmers lack the incentive to produce larger quantities of products than can be used by their own families and perhaps their neighbors. By distribution channels, I mean not only transportation, storage, handling, and processing facilities but also reliable markets. Reliable markets mean customers with the ability to buy farm foods at prices high enough to give farmers a fair return on their labor. When it pays to increase food production, farmers generally seek ways to do it. This was the case in the United States during World War II, when the demands for food at home and abroad gave farmers the economic incentive to adopt technology as never before.

It is significant that the countries in which food output per acre and per animal has shown the greatest increase are, almost without exception, the countries in which highly developed industry and concentrations of people in cities have provided farmers with industrial goods needed for efficient production and with profitable domestic markets for their products. Thus, favorable social, economic, and institutional conditions are essential for efficient food distribution just as they are for efficient production.

I recognize that some countries depend on imports to supply a large part of their food requirements. But, in total, only a small percentage of the world's food has ever moved in international trade. That trade cannot be expected to meet the needs of countries having little to exchange. I believe that the solution to food shortages in such countries must be found largely within the countries themselves.

In closing, I would like to reiterate the thought expressed in my opening statement. I am convinced that the world has the natural and scientific resources to feed its people today and for many years to come. I am equally convinced that victory against hunger will come only as nations and individuals acquire the vision, the will, and the spirit of cooperation necessary to plan and carry out so mammoth a task.

Water resources of the world

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IT IS A DIFFICULT TASK to say something significant about such a large subject as the water resources of the world in the short time that we have here this morning. In preparing my paper, I have assumed that you are interested primarily in the outlook for sufficient water to meet the requirements of the world's growing population, with special emphasis upon water requirements for food production and domestic use. The outlook cannot be portrayed precisely and simply because, to my knowledge, world-wide studies designed to provide such a picture have not been made. In the absence of such over-all information, we can do no more than render judgments based upon readily available data and individual studies of particular countries and locations.

I have divided my analysis into three major sections. First, I will make a few comments on the general outlook over the world. Second, I will examine the promise that science and technology offer for helping to meet future water resource needs. Third, I will suggest the probable impact of industrialization upon world water supplies.

THE GENERAL OUTLOOK

Implicit in Mr. Shaw's assuring statement that the food output of the world can be multiplied is the assumption that there is enough water as well as enough land to support such an increased output. My review of the data on the world's water resources confirms Mr. Shaw's estimate of the situation in general. There is plenty of fresh water to support a vast increase in agricultural output over the world. It has been estimated, for example, that in the case of India, which irrigates more land than any country in the world except mainland China, only 6% of the average flow of its streams is used for irrigation. In the United States of America net consumption of water for all purposes is only about 10% of average runoff to the seas. On every continent vast quantities of water flow into the ocean which might be diverted to food production. However, the situation varies widely. Some countries have enormous water surpluses which they will never be able to utilize for food production, at least. Others have already developed a major proportion of the resources within their boundaries. And since the value of water is quite low relative to its weight, it is frequently impracticable to transport supplies great distances to redress the balance between areas of surplus and areas of deficiency.

Australia is one example of a water-deficient land. It has been estimated that only 7½% of the area of that continent is suitable for cultivation and that the maximum area that could be irrigated annually is about 5 million acres (1). Once these lands are developed, increases in agricultural production are dependent upon more efficient use of land and water resources already being utilized, and upon the possibility of increasing the supply of fresh water through weather modification or desalinization of saline water sources.

A somewhat similar situation in some respects, yet different in others, exists in much of North Africa and Western Asia. Precipitation is low except in the high mountain areas. Cultivated agriculture is largely dependent upon irrigation. A substantial proportion of the total water supply is already being used. Population of the region continues to increase at a rapid rate. Although in most of the countries of the region there remains significant unused water supplies, the total supplies available for new development are quite limited. Egypt, for example, is dependent almost entirely upon the water resources of the Nile for its cultivated agriculture. To make better use of this historic source, the high Aswan Dam project is being built which will increase the irrigated area of Egypt by about a third, and will permit perennial irrigation in place of flood irrigation in Upper Egypt. Yet it is significant that a United Nations report estimates that the increase in agricultural productivity resulting from this project can be expected only to keep pace with the increase in population (2).

Water resources problems, however, are not limited to countries generally deficient in total water supply. India, for example, is generously endowed with rainfall. Nevertheless, large areas are arid and much of the country suffers a hot dry season when the land is parched and irrigation is essential to agricultural production. To increase the food supply for its growing population, India has initiated a series of ambitious irrigation schemes. Programs begun under the First and Second Five-Year Plans, upon completion, are expected to provide water for about 38 million acres. To realize this potential, the Indian Government estimates that an investment of approximately 14 billion rupees will be necessary (3). The point to be emphasized is this. Even in countries with large potential sources of water supply, the least costly supplies are quite naturally developed first. The application of additional water to land often

does not come easily. Storage and distribution facilities must be built, and these require careful planning and large investments of capital and labor.

In recognizing these problems, I am not pessimistic about future prospects. But there are difficulties ahead. In some areas of rapid population growth, we are approaching the day when additional sources of natural fresh water will not be readily available for development. Also, in much of the world where increases in food production are urgently needed, relatively large capital investments in water management facilities will be essential to a significant expansion of cultivated agriculture. For the most part these countries suffer from a scarcity of capital and available capital is in urgent demand to expand industry and promote economic development generally, as well as to meet pressing food requirements. The task ahead is neither a simple nor an easy one.

THE PROMISE OF TECHNOLOGY

In recognizing that some parts of the world are confronted with water shortages, a question usually arises as to whether desalinization of sea water and/or weather modification may offer an eventual solution to the water problem. These possibilities have captured the imagination of people everywhere. In view of the tremendous strides that science has made over the past couple of decades, there is reason to hope that at some future date it will be possible to secure fresh water from the sea at relatively low cost, and to influence in some measure the location and quantity of precipitation. In considering these possibilities, it is well to keep in mind their present status of development. Under present technology, it would cost at least $160 in the United States to produce 12 inches of freshened sea water for one acre of land. Yet, $20 is considered the upper limit that a farmer can afford to pay for that quantity of fresh water, and generally a third or half of this amount is a high figure. In other words, desalinization of sea water is still about 8 to 20 times as costly as it must be for practical utilization in the United States for agricultural purposes. In low income countries the disparity would be even greater.

However, in many parts of the world there are supplies of so-called brackish water. That is, saline water which has only 5 to 10% as much salt as ocean water. Such supplies can be purified at much lower cost than ocean water. In fact, it now appears that these costs are low enough to be economic for domestic and industrial uses in some areas. Nevertheless, even for brackish water, desalinization costs are still several times the amount economically justified for agricultural purposes.

In brief, the search must continue for a major technological breakthrough before desalinization can supply water for food production. But, the rewards would be so great if the search is successful that an intensive effort is justified.

Scientists differ in their views as to the degree of success which has been obtained through efforts to increase precipitation artificially. Official studies in the United States have concluded that weather modification activities have only been clearly successful in mountainous areas where because of topographic conditions there are super-cooled clouds. In such areas it is estimated that precipitation has been increased as much as 14% above the amount that otherwise would have occurred. Three points merit emphasis. First, where increases in precipitation can be obtained, results are realized at relatively low cost. Second, where precipitation is naturally low, a percentage increase of 15%, for example, may not be very significant. Yet, where arid regions depend upon the flow from more humid areas, such a percentage increase within the humid area could constitute a substantial absolute increase in water supplies to a water-deficient region. Third, techniques of weather modification are far from perfect. It is still not clear as to the precise conditions under which success can be expected or exactly where the induced precipitation will fall.

While practical techniques for increasing the supply of fresh water through desalinization and weather modification have yet to be developed, science holds other promises which when taken together may offer equal or greater benefits. In arid regions evaporation from the surface of storage reservoirs may total 100 inches or more a year. Over the earth the losses through such evaporation are so large that, if avoidable, millions of acres could be irrigated with the water saved. Research aimed at reducing losses from surface reservoirs and at possibilities for substituting underground storage for surface storage (where geologic conditions permit) holds real promise of materially reducing water losses in arid regions. Still other research is designed to increase the productivity of the water being used. All too frequently known techniques for getting maximum returns from a given quantity of water are not being applied, either because farmers are unfamiliar with them or institutional barriers inhibit their adoption. There is good reason to believe than an increase in the efficiency of water use, through the application of known techniques and research into new techniques, may offer the best promise of balancing the supply and demand for water at an economic cost in most areas of the world. Such advances will depend upon a wide range of research in agriculture, engineering and the institutional aspects of water use.

THE IMPACT OF INDUSTRIALIZATION

Much has been said in the United States about the large requirements of industry for fresh water. It is my impression that there is widespread concern that as industrialization advances in the underdeveloped areas, industry will compete with agriculture for water with the result that food production will suffer.

Studies of water use in the United States indicate that industry uses a great deal of water, but actually consumes very little in comparison with irrigated agriculture. In other words, most of the water that industry

withdraws from underground or surface sources is returned to a water course where it is subject to reuse. For example, the Ohio River Basin, with an area of about 100,000,000 acres and a population of about 19 million, is one of the most highly industrialized river basins in this country. It has very little irrigated agriculture. It is estimated that in 1954 withdrawals from water courses for all purposes in the Ohio Valley—and these were largely municipal and industrial uses—totaled on the average about 22 billion gallons per day. Net consumption, however, was estimated to be less than ¾ billion gallons per day, or a little over 3% of total withdrawals and only .6 of 1% of the average total runoff of the river system (4). The significance of these data can be illustrated by a comparison with Egypt. The water consumed by cities and industry in the Ohio River Basin is probably somewhere between 5 and 10% of net consumption in Egypt, and Egypt has only about 15% more people than the Ohio River Basin. It is evident, therefore, that Egypt could support a large expansion in industry with only a modest reduction in the allocation of water to irrigation.

Industry not only consumes relatively small quantities of water in comparison with irrigated agriculture, but in addition it has considerable flexibility in the amount of water which it uses. Industry often uses water freely because water is available for the taking. However, where there is good reason to limit the use of fresh water, industry has many ways of minimizing its requirements. In some instances air fin cooling can be substituted for water cooling; salt water can be substituted for fresh water; the quantity of water used can be minimized through recirculation. In many ways industry has demonstrated that it can adapt itself to making a minimum quantitative demand upon the world's water resources.

The most significant impact of industry upon water is the effect upon water quality. In many respects pollution is the most serious water resources problem confronting the industrialized nations of the world. Generally it is not organic pollution, which sanitary engineers have learned to handle, but inorganic materials that constitute the most serious menace. New products and new processes mean that new compounds are constantly being discharged into streams. Many of these are not subject to the usual methods of waste treatment and I understand that frequently their effect upon people is unknown. In our efforts to increase food production, we may be creating another kind of pollution problem. Various pesticides used to fight insects and plant disease may eventually be washed into streams which are sources of domestic water. We still know too little about the extent of such pollution or the effects upon the human body when chemicals of this nature are consumed in small quantities over an extended period of time.

In short, the competition of industry for water supplies can be viewed with relative equanimity, inasmuch as industry generally consumes relatively small quantities of water and it has demonstrated the capability of adjusting to a wide range of supply situations. On the other hand, the pollution of water by our industrialized civilization is serious and may be one of the gravest water problems of the future.

CONCLUSION

In summary, the world water resources picture offers us hope and assurance as well as concern. Ample water is available to feed the hungry, to cleanse our cities, to supply our factories, to move our commerce, to produce electric power. But to realize these benefits, we must accept and meet a many-faceted and difficult challenge that will demand a great deal from many people in many lands. Inasmuch as scientific advancement will be essential to our success, much of the emphasis should be upon research—research to minimize the water required to grow a crop, research to lower the cost of desalinization, research to improve techniques of weather modification, research in engineering to reduce water development costs, research to minimize pollution, and research to improve human institutions affecting water use and management. But other areas of endeavor will be equally important. There is the enormous task of education, of breaking down the barriers of tradition, so that what is learned can be applied by the farmer, the householder, the factory owner. There is the task of the planner, the engineer, and the economist to design water development facilities, and to produce systems which will assure maximum returns from available resources. And for many of the world's river systems there is a task for the diplomat, because of their international character. The Indus, the Nile, the Mekong, the Tigris and Euphrates, the Niger, and many others—drain more than one country. The realization of optimum benefits from these resources will depend upon the wisdom of the international agreements governing their development.

This many-faceted challenge attests to the complexity of the modern world, including its most common compound, H_2O. Yet, it is but a small part of the very large problem confronting mankind—the problem of bringing population and resources into balance so that people everywhere may find life worth living.

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Socio-economic factors that limit needed food production and consumption

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PREVIOUS SPEAKERS have presented the statistics of world food production, distribution, and consumption and have emphasized the extraordinary disparities among human dietary patterns. The picture is a gloomy one since it indicates that the sum total of social and scientific progress to date has failed to provide an adequate nutrition for one out of two members of society. The prospective future explosive increase in the world population portends problems of much greater orders of magnitude unless drastic improvements can be achieved promptly. And the frequent statement that the universal application of modern technology could double or triple world food production, although comforting, is unrealistic. Blind reliance on science and technology for the solution of basic human problems is both unreasonable and dangerous.

More than 50% of the world's labor force is engaged in some aspect of agriculture. The less well-developed areas of the world are characteristically agrarian and in the least privileged areas the struggle for mere existence is so severe that the vast majority of the population is forced into practice of subsistence agriculture. This situation effectively prevents the production of surpluses which could be used to support other facets of social progress and economic growth.

As long as the world population was insignificant in terms of available resources the situation was a simple one, but as the numbers increased complications set in. The most striking of these has been the evolution of geopolitical patterns and today the world picture of political units is both kaleidoscopic and illogical. The range in size, available resources, population, and social and economic development among the nations of the world is fantastic, and neither the political nor the natural conditions within these enormous extremes can readily be altered for calculated benefits. Individual nations can develop within the limits of population, climate, topography, and available renewable and non-renewable resources. Those countries with great natural wealth and relatively small populations have been able to create the highest known standards of living which are compatible with human dignity.

The temperate regions have in modern history presented the greatest attraction to mankind as sites of human habitation. As they have progressed man has learned a great deal about ways in which to improve his standards of living. In so doing, he has found ways by which to utilize more fully the resources of the tropics and the subarctics. The modern technologies developed by industrial societies comprise the greatest single resource for increasing world food production. This has become increasingly important in support of programs designed to speed the rate of economic progress in many of the less well-developed countries.

An international study of the socio-economic factors limiting food production and consumption reveals great similarities in patterns prevailing in widely separated areas. These reflect evolutionary stages in social development within the several countries. Each is the product of the interaction of natural phenomena and people. In land management the classic but destructive shifting system involving clearing, burning, planting, and eventually a move to a new area and a repetition of the process is widespread. Elsewhere land may be laboriously tilled with the traditional Egyptian plow, the planting stick or iron hoe, resulting in an inadequate seed bed and low yields. Hillside cultivation is an ancient practice in many countries which in its highest form consists of ingenious terracing and in its lowest of crude land preparation for two to three years followed by soil erosion. Pastoral systems are common and where pressures on the land are heavy, overgrazing is a common phenomenon frequently accompanied by sheet erosion.

Similar landholding patterns are repeated throughout the world. Where populations are sparse and resources substantial, precise ownership systems are lacking. However, as population pressures increase or as new elements enter the scene, several types of ownership emerge depending upon local circumstances. These may be tribal or some form of communal ownership; the latifundia under which great expanses of agricultural soils are held by the elite of the community with the bulk of the population relegated to the role of a labor force; large numbers of small or medium-sized landholdings and at the lower extreme the division and sub-division of land parcels until individual cultivators are forced to till one or several minute plots of land often quite distant one from another. This system represents one of the lowest

efficiency levels but is difficult to change where it is brought about by deeply rooted traditional systems of inheritance, credit practices which approach usury, and the treatment of land as patrimony rather than as a resource.

The management of biologic species by agrarian societies is critical to their progress. In many locales there has long been a process of selection resulting from a combination of ecological conditions with empiricism. Most commonly food crops and domestic animals are indigenous or long ago introduced species which are well adapted and productive under local conditions. Plant food species are primarily the cereals, but other high-carbohydrate food crops predominate where cereals are not well adapted. Rice, wheat, maize, sorghum, the millets, rye, and barley are principal foods in the cereal-producing areas, but elsewhere cassava, the sweet potato, potatoes, coconuts, and bananas are basic foods. Although over 3,000 plant species have been used for food and over 300 are widely grown, only about 12 furnish nearly 90% of the world's food.

A common phenomenon is the line selection of crop plants in response to local conditions. These may be low soil fertility, drought, or the incidence of disease. And over the years there has been a tendency to select domestic animal types which will persist if not always thrive under local conditions. Heat tolerance, resistance to pests and pathogens, along with the ability to utilize low-grade forages are among the most common characteristics of animal species in less advanced agricultural societies.

The social habits of communities in under-developed areas vary widely but fall into several patterns which may be repeated from country to country even though their origins are unrelated. Essentially all societies have experienced periods during which superstition dominated local customs. The response of the human mind to mysterious natural phenomena is historically well documented and it is disconcerting to realize the degree to which superstition and mysticism still pervade a major sector of the world's population. Although there may be great variety in local beliefs and customs, the similarity of the cause and effect is frequently apparent. Mysticism ultimately gives way to empiricism which ideally should be replaced by the scientific method. Unfortunately, however, this stage in the evolution of human thought is neither universal nor even predominant in terms of total numbers.

Local beliefs dictate customs and these include personal, food, work, and social habits. Again the patterns repeat themselves in widely separated areas with minor dissimilarities. The use of drugs such as opium, marijuana, hashish, peyote, and cocaine have originated independently in different cultural groups although these habits have become widespread through migration. The insistence upon certain foods for reasons of health, ritual, or taste preference is a well-established phenomenon. Work habits and marital and family patterns have many counterparts in social groups which have not had previous contact.

All of the foregoing factors have their impact upon food production and social and economic progress. They sum up to the conclusion that today the greatest cause of malnutrition is underproduction. Each agricultural unit which does not yield its full production potential represents a permanent irreplaceable loss to human nutrition and welfare. This loss may derive from unsatisfactory soil management, the use of low yielding food crops and animals, attack by pests and pathogens, and the lack of storage facilities, transportation facilities and markets for the preservation, distribution, and exchange of surplus commodities.

There is another form of underproduction which is even more serious than the material. This is the loss to society represented by the inability of millions of people to apply their total potentials to the improvement of standards of living individually and collectively. Just so long as there are these vast numbers whose opportunities for intellectual development are severely limited, it will be impossible to meet the increasing demand for food and other human requirements by the expanding populations of the future.

In dealing with world problems of health, nutrition, and technology, the scientist and engineer are able to establish specific goals and design projects leading to their attainment, but ultimate success must be predicated upon the skill with which the social sciences and the humanities are integrated with the physical, biological, and engineering sciences. Each day it becomes more imperative that the problems of the underdeveloped portions of the world be considered in their total context and that efforts to ameliorate undesirable situations be established on an interdisciplinary basis calculated to combine advances in science and technology with those in economics, sociology, and the humanities.

The most formidable barrier to further social and economic progress is the low level of education of peoples in many parts of the world. Education is the key that unlocks the door of understanding and provides opportunities to apply human ingenuity to the solution of social problems. Reasonable progress in bringing the less privileged members of society to acceptable standards of living can come only as rapidly as the educational process is extended and intensified. And progress in education must be matched by increasing opportunities for useful employment so that social progress and economic growth may proceed in concert.

There is no short cut to education and understanding although it is possible to intensify educational and training patterns in order to speed the rate at which levels of education are raised. However, regardless of the magnitude of effort there will inevitably be a lag between its application and the desired achievement. The human misery resulting from malnutrition, disease, and social patterns built upon ignorance, will persist, although

hopefully on a diminishing scale, for many years into the future. The persistence of all the old problems along with the introduction of new ones, as well as the increase in population, clearly indicates that concerted and enormous effort and substantial periods of time will be required to gradually ameliorate if not eliminate existing situations incompatible with human dignity.

In the past there have been many instances of population pressures becoming explosive with the resultant strife and warfare. At the moment, even though these pressures are already manifest in some areas and are definitely increasing, there appears to be greater agreement than ever in history that they can, and must, be resolved through the process of social, scientific, and economic development rather than conflict. However, unless rapid strides are made in education, science, and technology on an integrated basis, population pressures will ultimately pose a new series of menaces to world peace and all facets of human welfare. Although the threat to the future of society is clear, the solution is not apparent. However, certain essential elements to the solution are quite evident and may be stated as follows:

a) Agreement among nations as to the irrationality of the investment of massive resources in armies and armaments at the expense of human welfare.

b) Increasing international cooperation in all matters affecting the rational utilization of agricultural resources and the production and exchange of agricultural commodities, goods, and services on the most sound economic basis.

c) Herculean efforts in the field of education in order that new knowledge shall continue to become available in response to the needs of mankind and that more nearly all of the world's citizens acquire educational opportunities to permit them to take maximum advantage of available resources in support of decent standards of living.

d) An understanding of the threat posed by explosive population increases in the knowledge that sheer numbers can ultimately outstrip our productive capacity and at the same time create intolerable social problems. Serious and successful efforts must be made to stabilize populations so that we may not achieve a situation of chaos.

Freedom from hunger implies a great deal more than increased food production. Although food is the basic ingredient in support of life it is more importantly the source of the energy which enables mankind to take full advantage of its physical and intellectual potential. In the vital struggle to provide improved nutrition for the population of the present and of the future there must be included comparable efforts to broaden and deepen the understanding of increasing numbers of individuals so that as we win the battle with hunger, we will at the same time and as a part of the same effort win the goal of social progress.

Problems of food and nutrition—views and programs of FAO

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I FEEL GREATLY HONOURED to be invited to address the Fifth International Congress on Nutrition and to take part in this Symposium on "World Food Needs and Resources." The choice of this subject for your Symposium is another striking illustration of the concern that is being felt increasingly amongst thinking people all over the world about the inadequacy of current food supplies and the potentially dangerous situation that may arise in the future.

I am not a nutrition scientist myself, but am aware of the remarkable achievements of nutritional science during the last 30 or 40 years. The study of nutrition has brought to light facts of far-reaching importance to mankind. It has revealed how widespread malnutrition is in large areas of the world, and to what extent certain diseases and types of ill health can be directly attributed to it. People in many countries have contributed to the development of the science of nutrition, and taking part in this international Congress are many who have played a distinguished part both in the study of problems of nutrition and in initiating measures to solve these problems.

Today, more than a half of the world's population suffer from varying degrees of undernourishment and malnutrition. Undernourishment means plain hunger. Malnutrition has been called 'hidden hunger', an expression which implies that people who have enough to eat may nevertheless be unhealthy and even become seriously ill and die because their diet does not provide all the elements needed for satisfactory growth and health.

One of our primary tasks is to evaluate the present food production and consumption at national regional and global levels. In the last few years, FAO has conducted food consumption surveys in 26 countries, and regional nutrition surveys in Africa and Central America. It has also conducted two world food surveys and published the findings. It has made evaluations of nutritional requirements in terms of calories, proteins and vitamins. It has set up techniques for dietary surveys, particularly in the less developed countries which lack the means to pay for expensive surveys and where surveys of actual consumption are more urgently required because of the lack of data on production.

The knowledge thus obtained of existing food consumption and nutritional requirements makes it possible to assess deficiencies in diet in different regions and countries and in sections of the population within countries. The next step is to indicate the changes in food supply and consumption needed to make deficient diets more satisfactory for health. These changes must be brought about by well-conceived national food and nutrition policies. In most countries with low nutritional levels, it is essential to increase food production selectively. FAO has given direct assistance to some countries in setting up food production targets; for instance, in Central America, both as a group with an integrated exchange economy and for each country individually. The recommendations of FAO experts have directly influenced food production policies in some countries of this region.

When we consider the expansion of food supplies to meet world needs, we are confronted with the rapid growth of world population. It is obvious that if population continues to increase at the present rate, very large additional quantities of food will be needed, even at present consumption levels. It has been estimated that the present world population of 3,000 million will double itself by the end of this century, i.e. in another 40 years.

The Second World Food Survey showed that to increase the quality of the diet in terms of the original calories, it would be necessary to double agricultural production between 1946 and 1960. We now know that these targets have not been achieved except in a few countries which are already faced with unmarketable surpluses. In most countries where supplies are not sufficient to provide a satisfactory diet, the increase in food production has only barely kept pace with the increase in population.

The causes of undernutrition and malnutrition are numerous, but the major causes are poverty and ignorance. In large areas of the underdeveloped world, millions of people live on what they grow on their land. Their food lacks variety and many of those elements which constitute a balanced diet. Subsistence economy and malnutrition go hand in hand. The initial effort must, therefore, be concentrated on increasing agricultural productivity so that subsistence economy may yield place to a market economy. The surplus over consumption must produce the additional purchasing power needed to supplement the deficient diet. In order that the rate of growth may be sustained in the agricultural field, it is essential that agricultural development forms an integral part of balanced economic

and social development embracing industrialization, development of communications and social services. While economic development proceeds, it is possible to improve nutrition within existing income levels by spreading nutritional knowledge. The economist, the agronomist, and the nutritionist must work together to bring about improvements in nutritional standards.

The technical possibilities of increasing yields from land already under cultivation are tremendous. Among the means of achieving increased production are: the better use and control of water; the building up of soil fertility; plant breeding and seed improvement; reducing losses caused by pests and diseases; better farming equipment and management and more efficient agricultural services; the breeding and improved feeding of livestock. According to FAO specialists, production could at least be doubled in many countries through these methods alone.

The technical possibilities are not confined to yields from land only. The possibilities from scientific development of fisheries in the sea, thereby increasing world supplies of good protein and helping to eliminate protein malnutrition, are no less important. We are told by experts that we are at present harvesting barely one per cent of the food resources available in the sea.

Protection of food from damage and destruction is another big problem. We know that food and industrial crops are destroyed by pests and diseases in certain countries—sometimes as much as 25%. This wastage is preventable by the control of pests and parasites and by food processing. The underdeveloped countries still follow the traditional methods of processing, e.g. salting, drying, smoking, pickling, and fermentation, at community and family level, which do not require much material assistance but mainly demonstration through extension services. In FAO we place great importance on the progress that is being made in such advanced fields of research as atomic irradiation. We are also developing our work in refrigeration and canning.

However, it is one thing to point to technical possibilities; their realization is another matter. Many economic, social, administrative and political problems must be solved to enable scientific knowledge and techniques to be successfully applied. It is against this background that the Freedom From Hunger Campaign was launched by FAO on 1 July this year. The Campaign will attempt to focus world attention on the problem of food supply for the growing population, and to stimulate national and international action for raising agricultural productivity in the underdeveloped countries as a means of accelerating economic development and providing the adequate and balanced diet needed for a healthy life. The United Nations and its Specialized Agencies, national governments, non-governmental organizations, religious bodies, foundations, citizen groups, men and women of goodwill everywhere—all have been invited to participate and contribute their moral and material support. The role of FAO would be generally that of a catalyst and coordinator of these worldwide efforts. Any activity that would serve to swell the tide of this world movement would be regarded by us as part and parcel of this Campaign.

The Campaign is not intended to replace any programs at present under way for the improvement of food supplies and better nutrition. It is intended to supplement and reinforce programs which governments and international agencies are already carrying out, to create a better climate of opinion in which these programs will work with greater effectiveness. The basic idea behind the Campaign is that if there is to be a real breakthrough in the economic development of underdeveloped countries, the primary effort must come from the governments and peoples of the underdeveloped countries themselves, the developed countries supplying the marginal though crucial help in capital investment and technical knowledge.

The activities of the Campaign will fall mainly under three broad categories: *1*) information and education, *2*) research, and *3*) action programs. The information and education program would promote better knowledge and interest in the problem, and create, through appropriate studies and use of public information media, world-wide support for the objectives of the Campaign. A most important part of the education program will be to secure the voluntary participation of farmers themselves in the various activities under the Campaign. The experience in many countries has demonstrated that in increasing and maintaining productivity in agriculture the incentive of ownership and freedom of choice cannot be substituted by compulsive methods.

The research program under the Campaign will have two main aspects; firstly, the study and analysis of scientific and technical information that already exists in the related fields and of the possibilities of their practical application to immediate local and regional problems; secondly, long-range research aimed at developing new knowledge and new techniques which may lead to the opening up of new resources and will thus be of wider application. Basic causes of low productivity and the methods of eliminating them could, for instance, be studied under the first category of research programs. Long-range research should be concerned with a deeper study of biological and industrial resources and the human problems involved, and should include maximum use of solar energy and soil water; investigation into unused plant resources; determination of the true biological value of animal protein in the human diet and its possible replaceability by plant protein; potential further use of chemical and organic fertilizers; and similar problems. It should also include methods of putting to proper use some of those vast areas of the world, such as the Amazon Valley, which await investment based on research.

The action projects will be carried out mainly within individual underdeveloped countries, and will depend largely upon increased exertions by the governments and peoples of those countries. Since resources in

trained personnel and finances are limited, concentration upon a few key projects is bound to be more effective than their dissipation over many tasks at once. Each country will need to assess carefully its present position with respect to which types of additional activities under the Campaign promise to yield the best and most lasting results.

What contribution can nutritional science and nutrition workers make in the broad field of effort? The need to promote wider recognition of the importance of nutrition by governments and peoples in many parts of the world is clear. Even with existing food supplies, much can be done to improve food consumption through education. There is still considerable wastage in many countries, and too often food habits prevent proper utilization of certain foodstuffs. This applies particularly to the most needy groups, i.e. pregnant women and nursing mothers and young children; and it is always to these groups, unfortunately, that the numerous taboos which prevent utilization of protein-rich foods apply.

Nutrition education campaigns have been started in a number of different countries, on the one hand to increase general consumption of certain protein-rich foods such as fish, eggs, grain, legumes; and on the other to educate the mothers through 'Mother and Child Health' centers; through women's clubs; and younger people through schools. In the beginning, these were mainly relief programs consisting of the distribution of skim milk and sometimes vitamin tablets. Efforts are now being made gradually to replace the imported foods by local foods of equal nutritive value. This calls for the education of the producer as well as of the consumer.

I have already referred to the fact that we do not know enough about the qualitative and quantitative food consumption patterns of large communities whose nutritional levels we are seeking to improve. No rational food policies can be evolved unless these gaps in knowledge are filled. The national food balance sheets prepared under FAO direction and sponsorship are of considerable value, but they need to be further improved in accuracy and extended to more countries. Also, while they show broad trends in consumption, they do not bring to light differences in consumption in different groups within countries.

The Third World Food Survey planned by FAO will bring the world picture in relation to nutrition as up-to-date as possible on the basis of available data. This will no doubt serve a useful purpose, but will have to be supplemented by consumption surveys in those areas for which no reliable data are at present available. In this field, as in the other spheres of economic and social development, there is much room for coordination of national and international efforts. I am happy to say that some of our sister agencies have responded enthusiastically to our call for cooperation in the collection and correlation of basic data.

Both in research and action programs of the Campaign there will be a demand for trained nutritionists at all levels. The number of nutritionists, especially in underdeveloped countries where the need is the greatest, is pitifully small. The United Nations agencies concerned with nutrition are now giving increasing attention to this question, but the governments themselves must do more if real progress is to be made.

The Freedom From Hunger Campaign will run for 5 years. In 1963 there will be a stocktaking of the results so far achieved at a World Food Congress to be held in Rome. It is obvious that within this brief period all that we can hope to achieve is to lay a secure foundation for sound policies and practical action towards that ultimate aim. War, pestilence, and hunger have been the three historic enemies of mankind. Recent advances in medical science, supported by large-scale public health measures, have won significant victories against epidemic and endemic diseases. A global war today with the employment of nuclear and thermo-nuclear weapons could not be contemplated by anyone who is even remotely interested in the survival of the human race. But hunger still remains a major threat to mankind, and will be increasingly so in the future unless determined efforts are made on a world-wide scale to bring it under control.

The Freedom From Hunger Campaign is an attempt—the first attempt of all the nations working together—to face up to this challenge. The challenge can be met if there is cooperation from all concerned. Scientists especially will have a significant role to play. May I appeal to the nutrition scientists who have gathered here today, and through them to their colleagues all over the world, to do their part and help to bring closer the goal of a world free from hunger and malnutrition, which is, in fact, the very basis of their science. This is a job that must be done—can be done—and will be done—to ensure the survival of the human race.

AFTERNOON SESSION

Opening remarks

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THE MORNING SESSION of this symposium has dealt with the food problems and needs arising from the rapidly increasing population of the world, and the heavy demand these increasing numbers will make in the future upon the world's soil and water resources. Rapidly declining death rates in many countries, and the tendency for high birth rates to persist long after death rates have fallen, make for a dark picture in the field of future population increase. Moreover, while there is every reason to believe that it would be possible to raise food production sufficiently to feed the greater population which is expected in the next decade or so, the fact remains that fifteen years after the end of World War II about half of the world's people are still said to be inadequately fed.

Our attention this afternoon will focus on the difficulties and problems involved in the attempts to define, and if possible to solve, some of the world's major nutrition problems. This calls for direct action by physicians and nutrition workers as well as general programs designed to raise levels of living by increasing economic production.

The effort in which the United Nations and its specialized agencies is engaged, in the form of technical assistance, gives striking proof of the fact that providing more and better food for all the world's peoples makes heavy demands not only on soil and water resources but also upon man's inventiveness, intelligence and resourcefulness.

Major nutrition problems in the world today vary according to the conditions existing in the different countries. In the so-called underdeveloped countries, as you will hear, protein deficiency is a major problem, while in the highly developed industrial countries of the West, obesity and the clinical conditions associated with it are coming to be regarded as major problems of nutrition. The editor of the (British) Lancet has postulated that 1) disease prevents civilization, 2) that civilization prevents disease, and 3) that civilization also causes disease. In defense of his third axiom he writes,

"We keep forgetting that in the past few thousand years, and especially the last hundred, we have utterly transformed the environment, the habits and especially the food of our species; and we have put an appalling strain on the adaptability of a creature which Evolution had fitted to withstand the different circumstances of the previous million years. Such a change was bound to bring trouble—physical and mental. And I think the time has come when we should stop being complacent about the undoubted merits of our particular civilization, and recommending it indiscriminately to other people, and turn more of our attention to the damage it has done, and is doing. Often, by taking thought, this damage can be corrected; and here, I suggest, is not only a continuing but a fresh need for preventive medicine—for more not less of it. Prosperity is not enough; and high living may often prove to be bad and dangerous living."

All nutrition problems are of world-wide interest because of their social and economic implications, and no major nutrition problem can be isolated within the political frontiers of a single state. But nutrition is of universal interest and concern for other reasons as well: for it enters into every aspect of national existence, and it is involved in virtually every relationship between the nations.

Recent advances in our knowledge of nutrition have been little short of astonishing. The means which brought about these advances are stronger, and better organized and financed, than ever before. I mean, of course, the research workers and research institutions concerned with the different aspects of biology. We can look forward with confidence to greater advances in the future, because of greater numbers of better trained workers, more well-financed and equipped institutions, and growing belief in the promise of research to reveal more of the secrets of nature.

As regards population problems, the first World Conference on this subject took place at Geneva in 1927. A subject that was of interest to only a few workers thirty-two years ago, has now become the concern of sociologists, statesmen, writers, and groups of intellectuals in every country.

Although it is common in sophisticated societies to belittle the machinery for international cooperation which has been built up in the last forty years, I can think of no accomplishment which gives greater evidence that man is capable of remaking the world to his own liking. The entire apparatus for international cooperation has been built up in the last fifty years, and it is developing today at a faster pace than ever before. Confining ourselves to the twin subjects of population and nutrition problems, we note that the United Nations possesses a population branch of the Secretariat, a commission on population problem, and two international

demographic centers located in Bombay and Santiago, Chile, respectively. Population policies are prickly subjects to deal with internationally, and it is something of a triumph that an official international agency finds it possible to debate and to publish widely the facts of population change as they are known today.

However the field of food and nutrition involves a much greater number of international agencies. Beginning with several organs of the League of Nations, international concern with food and nutrition problems, intensified by World War II, manifested itself in the Food and Agriculture Conferences at Hot Springs and Quebec, the UNRRA Conference in Atlantic City, and somewhat less directly in the establishment of UNICEF and of WHO. From the first two Conferences sprang FAO and UNRRA, the latter a temporary agency which because of its emergency character was able to create valuable precedents for international action. These are only two of the intergovernmental agencies involved in world problems of food and nutrition. Others include UNICCF, UNESCO, ILO, The United Nations Bank for International Settlements, The International Monetary Fund, The Central United Nations Organization and its Social and Economic Commissions, and all the bodies which concern themselves with technical assistance. Efforts of these are supplemented by bilateral agencies and by private foundations, and international and national voluntary associations.

Properly planned and coordinated technical assistance contributing to the economic and social development of the less developed countries is often the best means of solving major nutrition problems in those countries.

Dr. Frank W. Notestein, President of The Population Council, Inc., has this to say on some of the implications of restricted forms of development assistance,

"Some thoughtful people are beginning to question the value of international assistance in the field of public health. Curiously, their doubts are a tribute to the spectacular success of such assistance. They fear that continued assistance will reduce death rates so rapidly that the resulting population growth will swamp any effort to improve living conditions through economic development. They point out that public health measures are most effective, and the resulting population growth is most rapid, in the poorest and most densely populated areas of the world—precisely those regions where the need for improvement in economic conditions is most acute."

It is, of course, foolish to believe that we can put a stop to the use of modern methods of preventive medicine in any country. Communication is so rapid and copious in the world today that revolution would follow any such attempt. Our only recourse is to increase the pace of all-round social and economic development, and this means technical assistance and capital investment on a vastly increased scale. In particular it means technological modernization of both agriculture and industry, and these are strongly dependent upon health improvement and education.

In the long run the transformation of a country from a mainly agricultural to a highly industrial economy, accompanied by the changes in culture which are brought about by education and other social measures, means that values change as they have changed in this and other Western countries, so that smaller families become desirable. This has been followed by the adoption of measures for family limitation, motivated by the desire to give children greater opportunities for education and a chance to earn better livelihoods. Different methods of limiting offspring are presently available, but until changes in the economy and the culture make such changes desired and desirable, families are not likely to take advantage of them.

To put the matter in mathematical terms, as Professor Notestein has done, countries with annual rates of population increase running between 2% and 3.5% must increase their economic production at the same annual rates simply to avoid a deterioration in living conditions. All improvement in living conditions must come from gains in production in excess of the rates of population increase. I draw your attention to the UN's Special Fund, which under the leadership of Mr. Paul Hoffman is seeking to stimulate private investment in underdeveloped countries by surveying and reporting on the rich possibilities which exist. International technical assistance is at too early a stage to have acquired the necessary experience, volume, and momentum to make it fully effective. The world has yet to learn that devastating poverty, malnutrition, and allied conditions cannot live peaceably on the same planet with luxurious living and abounding riches. And let me add that highly industrialized and more prosperous countries have their own problems in the shape of underdeveloped regions which influence adversely the more prosperous regions to which their inhabitants migrate in search of employment and better living.

Health, in which nutrition plays a major part, is the most tempting of the premiums which the highly developed states have to offer their less fortunate neighbors. It is desired by all peoples, and its sharing increases rather than diminishes the health of the donor. Nor is it the subject of prejudice, fear, or other troublesome emotion. It is thus the ideal subject by which to introduce, little by little, all out economic and social development through international cooperation. Men and women of all races and religions can learn to work together peaceably in the pursuit of good health for all, and may thus become accustomed to work together in other essential fields.

Accordingly, a major task of nutrition workers and students of population problems is to emphasize the need to speed up the economic and social development of backward peoples at home or abroad so that family planning may be substituted for the high fertility which frustrates economic progress.

The challenge to nutrition and public health workers is to demonstrate that science can solve, as well as create, social and economic problems, and that it can make vast contributions to human welfare in spite of its potentialities for disaster.

Major human nutrition problems today[1]

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THE PROBLEMS that we have raised successively have been studied, according to their importance, in the different sessions of this Congress. But the Scientific Committee of the Congress has thought that a panoramic view would be of interest. I must thank the Committee for the honor it has done me in charging me with this task, the difficulties of which I am well aware. The views that I am going to express are those of a physician trying to establish the balance sheet of the principal problems that are posed today in the science of human nutrition, pure and applied.

THE TOO-HEAVY AND THE TOO-LIGHT DIET

Today the problems of human diet and of its nutritional repercussions are oriented around two poles: over-eating and under-eating, too much and too little. Man is either over-nourished or not nourished enough, in both cases the result having serious, often deplorable, consequences on his state of health. In the scale of seriousness, under-eating appears to bring with it more disasters in the present situation of the world.

Let us take first the case of populations that are abundantly provided for. "Man often digs his grave with his teeth." It is rarely disputed that excessive plumpness—obesity—generally predisposes man to a series of mechanical, metabolic, and vascular complications that have the effect of shortening life. An excess of calories in the diet which comes with the consumption of too many lipids, too many carbohydrates, and too much alcohol also leads to a variety of pathologic states. And we ought to teach our acquaintances the role played by food intake in the etiology of diabetes, gout, lithiasis, hypertension and, above all, atheromatosis.

Artificial lactation assures the child a rapid body development. People are entranced with the possibilities of doubling the birth weight in 3 or 4 months instead of the classical 5. The biggest children are not the strongest. Is there a real advantage in increasing the rapidity of structural growth and weight and of ending in a more precocious puberty? The insufficiently checked consumption of certain nutrients on which advertising orients the mind can lead to over-dosages; thus it has been written that in this country that welcomes us so magnificently, hypervitaminosis A is less rare than the lack of vitamin A (axerophthol).

In these populations that are abundantly provided for, certain groups still remain affected. The notion of deficiency is by no means barred. Rickets persists in some form, more attenuated, it is true, than formerly; it is not rare to observe old people with nitrogen deficiency, more or less masked. And how many of these cases labeled neuro-vegetative dystonia do not reflect camouflaged hypo-vitaminosis of group B? Here one returns to non-clinical or subclinical deficiencies.

In normally developed regions alimentary problems, in the strict sense, do not in fact any longer exist. What remains to be done is a problem of popular education, of better distribution among the social classes, and also of distribution to reduce the parasitism of intermediaries.

In underdeveloped countries the problem is more poignant. It is that of scarcity, in the solution of which each of us has a responsibility. There are several hundreds of millions of people who do not appease their hunger in some countries where only a small minority are properly nourished or even over-nourished. In this global alimentary insufficiency, it is, in the highest degree, the lack of proteins which dominates, and from afar, following more subordinately, the deficiencies, especially vitaminic. There are territories where edema of malnutrition and kwashiorkor, proof of nitrogen deficiency, are almost the rule; elsewhere, or associated, are beriberi, pellagra, avitaminosis A, etc. The magnitude of the task to be performed in nourishing these populations is rendered more immense by the rapidity of their growth. It is one of the preoccupations of the world today. Also, all the interested governments and the great international organizations (FAO, WHO, UNICEF, etc.) are endeavoring to set up adequate measures: development of local resources, in the sense of the production of nitrogenized elements of high biological value, and economically the least expensive; also, the rational utilization of these by a better popular education. It is towards a better knowledge of the valuable protein sources that research in nutrition, already leaning in that direction, should intensify its effort. This is a problem of first magnitude, for the great hunger of the world is a nitrogen hunger.

ARE ALIMENTARY STANDARDS ADEQUATE?

One thus sees humanity enclosed between two extremes, and the dangers of a diet too abundant or too poor. The great problem which the nutritionist faces is to establish an alimentary standard which will serve as a frame of reference. Those which we have now, for lack of better, and in spite of the services they have rendered, appear to us disputable. Daily requirements—can they really be defined? They vary all the time. One can

[1] Original text in French.

establish average standards valuable for a group, but it is quite different for an individual. Our alimentary tables indicate precisely the daily quantities recommended. Would it not be logical, rather, to fix certain limitations not to be exceeded, either above or below, and to better determine what can not be done?

The next few years should permit the definite elucidation of the question of vegetarianism. For many of us the vegetarian diet is considered nutritionally inferior. The child often enough shows a repugnance vis à vis food that education has not overcome. Atheromatosis is less frequent in vegetarian populations. Might it not be the generally depressed conditions of life of vegetarian populations which are the source of the physiologically deficient states so common among them, and not the diet itself?

It appears also that one could reduce with advantage the value of certain caloric rates recommended in our tables. The existence of reserve body fat—is it necessary if man is assured the constant benefit of an appropriate diet? The lipid depot—is it not already some index of forcing the metabolic possibilities of the economy? Steatosis of the liver cell is a good signal of a nutritional disorder already patent. A better recognition of need appears indispensable and this also is important for the progress of pure nutritional science. Concerning energy needs, which one is superior for assuring coverage, carbohydrates or lipids? Does the body have a real need of the latter, besides the necessary quantities of liposoluble vitamins? And the need, the individual variability of which we have underlined, depends on the state of previous nutrition, the present diet, and also on exterior factors such as modes of dress, heating systems, etc. One can certainly pride oneself on the progress made towards a better understanding of the metabolism of proteins; but new work should give more precisely the necessary quantities of the different amino acids. This problem will not be easy to resolve. These determinations will be difficult because they depend on the context; the quantities of amino acids to be foreseen vary with the nature of the diet. One finds himself in the situation of establishing the validity of the mixtures, but the interrelations are very tight—when an essential amino acid is lacking, this is certainly a limiting factor; on the other hand, if there is an excess of it, there is an impairment in the utilization of the whole ensemble. The more we advance in the study of internal metabolism the more we see the diversity of the reactions in which amino acids participate. The deficiency of one of them not only functions like a limitation of the tissue's synthesis, but it also has metabolic repercussions which appear more or less successively.

The study of salt requirements has been somewhat neglected. What definite criteria do we possess of a requirement of 12–15 g of NaCl per day? What is the role of routine or of ancestral habits? While we know quite well the energetic and nitrogen pattern in the tissues, we are quite ignorant about the minerals. What is actually the indispensable amount of each ion? A study similar to those made on vitamins should be conducted for each of them: the possible effects of a diet containing all known nutrients except the ion under study should be studied. And the interpretation should be very subtle. The fact of maintaining all variants constant, with the exception of one, tends to make it appear that the variable under study has an action which is independent of the other factors. Yet the dietetic and nutritional interrelations are such that this fact cannot but be exceptional. Here there is again the need of trying to understand the phenomenon in terms of balances.

As for vitamins, it is important, as Terroine has phrased it, to suppress the "current divorce between the biochemist and the physiologist or the physician." The knowledge of the chemical functions of vitamins does not clarify in general the physiological phenomena of nutritional deficiencies. The chemist, for instance, states that biotin is the carrier of hydrogen, but how does this explain hair fall-out in animal avitaminosis? The cooperation between scientists in different fields is still a must and should be encouraged. Can we still speak with validity of a ponderal vitaminic need on its own? It depends on the context, on the caloric value of the diet, on its pattern and coverage of glucides and lipids, on the nature of the mineral contribution, on the condition of the gastrointestinal tract and its repercussions on the absorption, on the condition of the receiving or utilizing viscera. It seems dangerous to determine an absolute value; it would be wiser to look for a balance, a notion on which Madame Randoin and Simonnet in France have insisted for a long time. Niacinamide seems to be required only in cases of tryptophane deficiency. The addition of a nutrient can elicit the deficiency of another factor by neutralizing its effects. This is perfectly established in the case of the anti-vitamin action. The multiplicity of interreactions seems to be boundless.

NUTRITIONAL PATHOLOGY AND THEIR LIMITATIONS

In the field of nutritional pathology we find the same extraordinary complexity. Let us take the case of experimentation on these major problems of atheromatosis and cholesterolemy. We see that the nature and the amount of fat, or protein and carbohydrates has a definite role, as well as the amount of cholesterol and folic acid, of natrium, calcium and magnesium (with their relationship), of vitamins B_6, E, C, D, of the ration of purine and pyrimidine substances, and, from the endogenous point of view, the thyroid gland, the sex hormones, and the bacterial flora of the intestine.

If we want to determine in a systematic way the role that each one of these factors plays in the etiology of atheromatosis, what a multitude of experiences we can have!

It is imperative to make nutritional and clinical surveys in the countries where nutrition is obviously defective. It is foreseeable that the knowledge of the principles of an adequate diet will be more and more

enlarged and that these exceptional fields of study will disappear. Scientific research has not yet drawn every advantage from these human groups; the Bantu populations ingest up to 200 mg of iron daily and suffer siderosis. The Nigerians, with a large content of palm juice in their diet, absorb great amounts of vitamin A.

The first half of the 20th century marked the disappearance of infectious disease as an important factor of mortality; in this epoch the major importance of nutrition in pathology emerges. Every disease has a nutritional aspect. There are pathological conditions of evident nutritional etiology: diabetes, goiter, hemosiderosis, lithiasis, nitrogen deficiency in infants and adults, avitaminosis, certain goiters and certain anemias, certain congenital malformations, etc. But every organic condition, whatever its nature—infectious, parasitic, traumatic, neoplasic and necessarily metabolic—is accompanied by a nutritional incident, which is more or less marked according to the individual case. Nutrition thus has relationships with all the branches of medicine.

The need of nutritional research is still great and boundless. But its interest should have as a limit the assurance of its safety, because due to reasonable enthusiasm the investigator can easily place the human body in danger under experimental conditions. Even with the risk of being taken as a retrograde, I think—and there are many who think it base—that the use of large amounts of tracing substances in man is not free of danger, even in those cases in which the Geiger counter is silent at the end of the experiment. We are not completely sure that certain protoplasmatic alterations are not detected and that they manifest themselves dangerously many years later. Certain experiments study the effect of an intake of antibiotics, during several months, on the development of normal children. We are, doubtless, moving towards an experimental human medicine which is almost animal. I think that a Congress like ours is the most appropriate place to call attention to this important problem. Perhaps the International Union of Nutritional Sciences would be able to investigate this problem within the several national committees, in order to have the opportunity of analyzing it in a future congress.

One aspect, especially important in nutritional investigations, is, we have said, that the conclusions are too quickly released through public and journalistic information always avid for news. As soon as it is believed that a scientific study can be implemented, it is made available to the public. They don't wait until more definite work has been done and more conclusions have been made, covering the great variety of nuances of a problem. Many times this type of commercial advertising adds a repulsive note. This danger, which threatens all the branches of medicine, is particularly acute in the field of nutrition.

APPLIED NUTRITIONAL SCIENCE

When one thinks that a solution has been found or at least that a problem is almost solved, should one believe that new developments cannot be expected? Let us take the example of dental caries. It is already some years that, on this side of the Atlantic, it has been believed that this problem is practically solved. And when the fluorine content of drinking water is found to be insufficient, fluorination begins to be a measure of classic public hygiene. What do we see today? The Swiss seem to be seriously opposed to fluorination for fear of the antagonistic action on the thyroid iodine as well as of the inhibition of enzymatic activity and of kidney injury. Some others think that dental caries is an epidemiological problem rather than a nutritional one, and the oversimplified theory that sugar is solely harmful to teeth is losing its backers.

The progress of applied nutritional science is not free of posing some new problems. Man's nourishment is modified. There is a tendency, more marked each day, towards a concentrated and canned form of nourishment. In the areas abundantly provided for, this tendency derives directly from certain decisive factors such as the taste of the public or the new forms of social activity: eating in the factory, working women, etc. In those areas poorly supplied, there is the need of maximal utilization of local production, of storage, and of avoiding all losses, as well as the need of importing food. Green vegetables become less looked for in countries with high standards and are usually neglected, through contempt, in under-nourished countries. The methods of storage which should be preferred are the physical ones and, among them, heat. Canned food appertised represents the ideal form of conserving.

The food industry is trying, however, to employ substances or edulcorates, all of which have a more or less similar anti-oxygen action.

Which criteria should be followed to authorize the addition of such substances to foodstuffs? This represents a major problem. It is indispensable first to define the principles on which we base our assurance that these substances are safe. Animal experimentation doubtless throws certain valuable conclusions, but how can we be sure that certain substances ingested in small amounts during prolonged periods of time do not have toxicity eventually? Every experimental system seems to be arbitrary. Doubtless it is indispensable to distinguish among the additives the natural components of the tissues, the elements of intermediary metabolism and the chemical substances as such. The addition of a nutrient can be in itself dangerous. A portion of lysine intravenously increases the urinary excretion of cystine, arginin and ornithin; the premature release of milk enriched with lysine brings about the conditions of a cystinuria.

The idea that the optimum requirement of one of these nutrients does not surpass the minimum requirements should also be re-examined carefully; the idiopathic hypercalcemia of the British population seems a consequence, in certain cases, of the ingestion of irradiated milk and of a milk too rich in vitamin D, in an excessive amount for certain human beings.

We see, thus, the immense field which is still left to investigation and all the studies to be undertaken to place the benefits at the disposal of man in the areas in which there is a scarcity of balanced and adequate foodstuffs.

Our statements could elicit great pessimism. But this is groundless. The intelligence and ingenuity of man are applied to discard or to overcome the obstacles accumulated by nature. It suffices to look back in order to realize that there is hope. Sixty years ago very few things were known about the relationships between health and nutrition. It was known that the proteins, the lipids, the carbohydrates and certain inorganic salts play a role, but that was all. Since then, 40 nutrients indispensable for life have been identified.

The 20th century has made of nutrition an autonomous science, well defined, with its pathological ramifications and its social and preventive imperatives of nutritional hygiene. Gradually, in the consciousness of governments and peoples, nutrition linked to alimentation has become an object of constant preoccupation and one of the primary factors of the peace of the world. The honor conferred upon us by President Eisenhower in opening our Congress is a remarkable confirmation of this. This public interest can constitute an exceptional incentive, but it also confers upon us, the scientists, a tremendous responsibility.

The prospect of meeting protein needs

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THE PRECEDING SPEAKERS IN THIS SYMPOSIUM have thoroughly covered the major nutrition problems of the world in relation to population and world food production. I would now like to direct your attention to a specific but vital aspect of this subject, namely, protein and the prospect of meeting the protein needs of the world.

But first let us look at some of the reasons why we should give special consideration to protein needs. The word protein was introduced by a Dutch chemist, G. J. Mulder, in 1839 as a term for a substance that was present in all plant and animal tissues. He said that it was "unquestionably the most important of all known substances in the organic kingdom. Without it no life appears possible on our planet. Through its means the chief phenomena of life are produced."—a remarkably astute statement for his time and one which is still valid.

In order to emphasize the vital importance of protein I would like to point out that proteins are *normal* constituents of all of the cells and fluids of the body with the exception of bile and urine. They are important in controlling the balance between cells and intercellular fluid, between tissues and blood, and in maintaining fluid balance. They are constituents of enzymes and hormones and are involved in immunological and allergic substances.

Of the three basic food substances—fat, carbohydrates, and proteins, it is only the proteins that furnish the nitrogen without which the growth, development and replacement of tissue is impossible. Without protein, death is inevitable. With an inadequate supply of protein, normal growth and development are impossible.

Proteins differ from carbohydrates and fats in another important way. All proteins are made up of a number of fragments called amino acids which are put together in an infinite variety of combinations. They are highly specific not only for different plants and animals but even for different tissues in the same organism. It is this specificity which largely accounts for our characteristics as individuals. Thus it is merely a slight difference in amino acid arrangement which changes normal oxygen carrying hemoglobin to a hemoglobin which results in anemia and death.

The human body utilizes protein by breaking it down into its amino acid fragments which are then either used for energy or are rearranged into new molecules suitable for the repair or building of muscle and other body tissues, hormones and enzymes. There are eighteen commonly occurring amino acids in our foods. Fortunately, the body is able to manufacture all of these except eight which therefore are called essential —since they are essential to man. Therefore the presence or absence of one or more of these eight amino acids in a protein determines to a large extent the value of that protein to man. This has led to the necessity for assessing the protein quality or the biological value of the various protein foods. It is not only essential that man have enough protein food but it also must have the right amino acid composition. These and other more complicated relationships in regard to protein foods have retarded the widespread recognition of the great importance of the problem.

Man, driven to eat by the sensation of hunger, becomes satiated when his caloric needs are met. A sense of fullness, warmth and contentment may lull him into a peaceful contemplation of the rumblings and gas production of his digestive processes. His need for protein however is not felt and goes unrecognized unless the deficiency is severe enough to bring him to famine edema and the edge of the grave while his children die of kwashiorkor or remain 'underdeveloped.' He does not see the reason why the herdsmen and the hunters and the eaters of high quality protein have always been the most vigorous tribes and civilizations. It is no accident that the underdeveloped people in the world today are those on poor protein foods.

Man has always tended to base his food production on his need for calories and his desire for money. Much of the agricultural planning today is based on increasing the yield of the same foods that are now being produced. It *is* essential that caloric needs receive first consideration because the gnawing hunger and emaciation produced by insufficient calories quickly leads to desperation and early death. However, this need for calories is so closely tied to the need for protein that the two must be considered inseparable from a practical point of view. I have mentioned that the body puts together its own

specific proteins from the amino acid fragments of the food proteins. This requires energy. Presumably the source of this energy is the high energy phosphate bonds obtained from fat and carbohydrate. It is well known that an insufficient supply of calories results in inefficient use of protein. This is also due partly to the fact that much of the protein may be utilized for energy if there are not enough calories from other sources.

Although our knowledge of the fundamental importance of protein foods to public health is relatively new and there is still much to be learned, it is quite evident that if we are to make real progress in the improvement of the health and well-being of mankind it is not enough just to urge the production of more food. Agricultural programs now must be planned for the production of more food of greater and better protein value. This enormously complicates the problem but just increasing the present staple crops of much of the world will only help protect the growing mass of humanity from starvation. It will not improve their condition in life.

One of the tragedies of protein malnutrition is that it strikes most severely at small children. This is because the protein to calorie ratio must be higher at this age than at any other time of life. Since growth is impossible without protein, the rapidly growing child must have more of it in relation to his weight than an older person or he will either be stunted in growth or die of kwashiorkor, which many scientists think is actually the result of getting calories from carbohydrate in the presence of a serious deficiency in protein.

The problem can be graphically illustrated by manioc or cassava which is a staple crop for many millions of people. Manioc contains a very small amount of protein (1.2%) and the little protein present is of poor biological value. Thus manioc, a starchy root, is primarily a source of calories without protein. It has many attractive qualities as an agricultural crop and as a satisfier of hunger, which account for its popularity. During the period 1948–1952 in Africa south of the Sahara the average production of manioc was 27.4 million metric tons comprising 27% of the calories and 37% of all of the main staple food crops, greater than any other main staple crop.

Thus to encourage further production of manioc may be merely a means of prolonging present conditions of serious protein inadequacy. A summary of nine selected dietary surveys of this area compiled by FAO shows that for seven of the groups the average annual protein intake per day was 59 to 65% of the requirement, although the calorie intake was 92% of the requirement or better. It is obvious that merely increasing the production of manioc will do little or nothing to improve the condition of these people. It would be much better to meet their calorie needs with an increased production of foods which will supply both calories and protein. The question is: what is the food that will do the job? No one can afford to tamper with a main staple food crop until a proven superior, acceptable, economical, agriculturally feasable replacement is available. And here let me insert a word of caution to nutritionists. One should never be *against* any food and I would strongly advise against telling any person not to eat manioc. Instead they should be taught the need for eating larger amounts of foods of better quality. Once they can get better quality foods the quantity of manioc eaten by the individual will be adjusted by his appetite.

Before we look at the possibilities of finding the necessary foods for which a desire can and should be created let me point out that the scientist in this field today has only solved the easy part of the problem when he succeeds in finding a new food product or mixture or in improving an old one. He then must tackle the infinitely more difficult problem of creating a desire for his product on the part of those that need it and of successfully introducing his product in the usual channels of commerce where it must successfully compete with products which may be more attractive though of less nutritive value. At the top of the list of preferred proteins from the biological point of view stand the animal products, eggs, milk, and meat; but these are expensive foods. They require relatively large land areas to produce a relatively limited number of calories and many areas are totally unsuited for their production. They are always highly desired foods except where religious prejudice forbids their use.

It has been amply demonstrated that enormous increases in chicken and egg production are possible under conditions of expert and careful management and it is possible that we may see the day when one pound of chicken can be produced from one pound of feed. Furthermore, ruminant animals may serve as converters of non-edible materials to high quality protein such as the use of cellulose, urea and molasses for cattle. Also the cultivation of rapidly breeding small animals such as rabbits could make great additions to our protein supply. The important point is careful management to make the best use of the available land in the most economically satisfactory way.

Here I also must touch briefly on the problem of distribution. It is not enough just to increase production. This is painfully apparent in the world today. Means must also be found to achieve better distribution in an economically feasable way, and I refer to local problems of transportation as well as movement across national boundaries. These problems are beyond the scope of this paper and I can only say that better distribution of the available supply of high quality protein would be of great biological importance if it could be made practical.

Man discovered long ago by observation what the present day scientist is confirming in the laboratory; namely, that a good protein improves the usefulness of a poor protein by supplying missing essential amino acids. Thus throughout the world we see the use of the high quality proteins extended by mixing them with those of lesser biological value. Spaghetti and cheese, bread and milk, rice and fish, chili beans and meat are just a few

examples. The most efficient use of high quality protein foods is obtained by mixing and diluting with a variety of other foods. Today, in the nutrition research laboratories of the world many scientists are seeking ways to extend this ancient practice to new mixtures of available foods that are now little used. Such as the use of milk protein mixed with peanut flour and ground meat mixed with cereal flours. Of all the animal proteins, fish offers the best possibility for large scale increase. Much land which cannot be used to advantage agriculturally can be converted to fish ponds and a harvest of good protein quality obtained. Irrigation systems and streams can be stocked with edible fish and the enormous fish resources of the ocean have not been explored in many areas. The seasonal variation in the run of fish and the rapid spoilage of the product have greatly retarded full development of this resource. Although dried, salted and smoked seafoods have been widely used, the technical difficulties and the expense of creating a satisfactory fish flour still retard this development. One can anticipate however that these difficulties will be solved and that greatly expanded production of high quality fish products can join the other animal proteins in being used in mixtures with products of lower biological value. However, the economic and transportation factors may still be serious obstacles to making these products very effective in relieving the protein shortage in many underdeveloped areas. These products really do not touch the heart of the problem in many places just as the movement of any kind of food from a highly productive high economic area can never solve the problem. The supplying of such foods may only support more of a rapidly increasing population which will require the movement of more food to maintain a larger and more dependent population without any improvement in status. The fundamental reason for underdeveloped populations is lack of productivity. Therefore, the solution lies in increasing productivity and in finding a source of protein that can be produced within the resources of the population concerned.

There is a large group of foodstuffs now largely wasted which immediately come to mind. These are the oil seed press cakes. These are produced as by-products of the vegetable oils and to a large extent are now used as fertilizers, animal foods or completely wasted. The most important of these products are peanuts or ground nuts, cotton seed, coconuts, seasame and soy beans. There are others of lesser world importance such as sunflower seed, which could become of considerable local value. Although they contain about 40% protein a large amount of these products are not available as human food either because primitive processing is so crudely done that the press cake is not fit for human use, or suitable processing methods for economical recovery have not been developed. The possible importance of these products is indicated by the fact that about 1.5 million tons of peanut press cake in India could be diverted to human use, and if the peanut press cake in Nigeria were all used for human food it would add 10 grams of protein daily to the food supply of every inhabitant of the country. Cotton seed is important in Mexico, Brazil, Egypt and India and is now being used in food mixtures in the United States. Coconuts offer a particular opportunity because of the enormous amount of copra which is wasted. There is no known way at the present time to make a satisfactory protein food from this product because of the technical problems involved. When these are solved another great source of protein will become available in the Philippines, Indonesia, and India. The technical problem of removing the hulls also handicaps the use of sesame.

The oil seed press cakes represent a wasted resource which can be immediately utilized, however since they are based on the vegetable oil industry they do not help very much in solving the problem in villages where there is no oil seed industry. So we must look elsewhere for additional protein resources. The next thing that comes to mind is the soy bean.

Soy beans have a very special interest because they have been for centuries an important part of the protein supply of China and other parts of Asia. They can be cheaply produced and adapted to a wide range of soil and climatic conditions. They contain enough oil (about 20%) so that they are used as an oil seed in some areas and the press cake utilized for food. They contain more protein than most legumes (40–45%) and can be made into a large variety of acceptable dishes. Although the protein is of better quality than other legumes, it is still inadequate in the sulphur containing amino acids. Soy beans may be regarded as a bridge from our soil seed press cake problem to the legumes, for the legumes represent the great class of vegetable foods to which we must turn for any real attack on the protein problem. There are a multitude of varieties of legumes and they are grown and used as food in all parts of the world. Although they vary widely in composition most of them contain about 20–25% of protein and they are the most important source of protein for vegetarians. The sulphur containing amino acids, methionine, and cystine are usually the most important limiting amino acids in the majority of legumes. In others the limiting amino acid is tryptophan. However, the legumes have a good lysine content which makes them particularly useful in diets based on the cereal grains. There are many problems yet to be solved in the use of legumes. They must be cooked in order to improve their nutritive value and digestibility, and many of them contain highly toxic materials which must be removed or destroyed before eating. A well planned research program can be expected to solve such problems and find legumes that are suitable and acceptable in many parts of the world.

Other possible sources of protein present greater problems and difficulties. Nuts are expensive and usually can be eaten only in limited amounts because of the high oil content. The cashew nut is unusual in its relatively high biological value and digestibility.

The possible use of the protein of green leaves such

as alfalfa or grass has received consideration because of the high yield of protein per unit of land per year. Such products have many drawbacks in color, odor and taste. It still remains to be shown that an economical, acceptable product of high biological value can be successfully introduced and widely used.

Algae have also been extensively studied with greatest attention to chlorella. Under proper cultural conditions a chlorella of 50% protein can be produced with a fairly good amino acid composition except for deficiency in the sulphur amino acids. Economic problems in production and acceptability make this still a highly experimental product.

Yeast has also received extensive study since it consists of about 30–40% protein which is deficient in methionine. It is acceptable only in limited amounts and therefore can be used only as a supplement.

Molds also synthesize protein of varying composition according to species, medium and growing conditions. They have been found deficient in methionine and tryptophan. The biological value is highly variable. They still must be regarded along with bacteria as highly experimental at the present time as possible sources of protein, although they may prove to be of great value in supplementing other proteins. Mold fermentation of soy beans and other foods has been used for a long time and studies are now under way to determine the nutritive value of such products.

Finally, we must give attention to the great possibilities that lie ahead in the chemical industry. Some essential amino acids now can be produced in commercial quantities at prices that make them practical possibilities either for animal food or human food. Although the use of synthetic amino acids for the purpose of making poor protein foods better is in its infancy it offers enormous potentialities for the future and even today could be utilized to advantage in improving the protein quality of some foods. Thus the addition of lysine to wheat flour greatly improves the protein quality.

The prospective world protein supply then consists primarily of: *1*) animal products, *2*) sea foods, *3*) cereal grains, *4*) oil seed press cakes, *5*) legumes. The animal products and sea foods must be diluted with cereal and vegetable proteins in order to get the greatest usefulness. However, it is important to recognize that without using any of these animal products adequate protein nutrition can be attained by suitable mixtures of cereal, legume and oil seed products.

One of the most interesting developments in this area has been the work of the research group in the Institute of Nutrition of Central America and Panama where a mixture of maize meal, sorghum, cotton seed flour and yeast has been shown, under conditions of careful study, to be satisfactory as a source of protein for growing children. Furthermore, this product has been put into commercial production. Another equally interesting project has been the development in India of a mixture of peanut flour and bengal gram (a legume) which also has been given careful study, and shown to be a satisfactory source of protein. It is now being used by school children with every prospect of continued increasing production and use. The development of other mixtures is under study elsewhere.

However, this is only the beginning unless this work can be followed by education of the poor and illiterate to make them understand that they must give these foods to their children in order for them to live and grow normally.

Although WHO, FAO and UNICEF have had joint programs in nutrition for many years, a new kind of cooperative program is required if the best progress is to be made in meeting protein needs because of the necessity for finding the answers within the productive capacity of the country concerned. This means national programs of broad scope based primarily on economic and agricultural resources with public health, education, and welfare going ahead simultaneously in a coordinated national effort with the vast resources of WHO, FAO and UNICEF used in a joint advisory capacity. Unless it is done jointly little progress can be expected.

For example, a continued increase in current staple crop production may be merely a means of delaying the onset of mass starvation. If we are to improve the status of the people of the world in longevity, health and productivity we must increase and improve the protein supply as rapidly as possible. This is feasable if we combine our planning with education that teaches that the satiation of hunger alone is not good nutrition, that foods have different nutritive values and that if our children are to live and grow normally they must have adequate protein. The problem is rapidly becoming more serious. One student of populations recently pointed out that although it has required from the dawn of civilization to today to produce the present world population, this population will double within a generation at its present rate of growth and the *rate* of population growth in the United States since World War II is higher than India or Japan and that if this present rate of growth continues in the U.S. for less than another hundred years the population of this country alone will be one billion, or more than one-third of the present population of the entire world. I quote these figures merely to emphasize the seriousness and the magnitude of the problem of finding enough protein for the world.

If we utilize now waste oil seed press cakes, if we make the maximum use of the products of the sea, if we turn from wasteful agricultural practices, if we improve our management of livestock, and increase our production of legumes, if we dilute our high quality animal proteins with those of lower quality, if we use our chemical ingenuity and capacity, we certainly can improve the condition of millions in the world today and delay the day when the world's supply of protein becomes more inadequate than it is today. For the

foreseeable future there is no problem in our protein supply that cannot be solved by education, research and by good planning until the day comes when the number of people simply overrun the capacity of the earth to meet their needs in many ways other than for protein.

Now I would like to conclude on a note of optimism. I feel that we can see a path ahead of us through the forest of problems which can lead us to the goal of making life better for millions of people, if we will only follow it with intelligence, diligence, perseverance and above all, good will to all men.

Food and protection of health

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MAN, UNLIKE PLANTS AND LOWER ANIMALS, need not rely on the whims of nature to provide his food in quantity and quality necessary for life and reproduction of his species. He has acquired over the centuries the knowledge and the means for controlling food to the point where it has become the environmental factor most susceptible to man's regulation. There is, however, much evidence throughout the world today of man's failure to exercise consistent and intelligent authority over this phase of his environment. Ideally, man should maintain certain equilibria or balances in his effort to regulate food and protect his health.

The first equilibrium is that of *quantity*. The maintenance of a constantly adequate intake of food energy for all population groups and for individuals within these groups, without insufficiencies or excesses, concerns alike political leaders and medical and public health workers. The second equilibrium is that of *quality*. Inadequate effort to ensure quality is potentially dangerous, whereas undue concern over it may be wasteful of resources, time, and money. The third equilibrium is that of *distribution*. Irregular distribution of available food leads to regional and seasonal fluctuations in the quantity and quality of food supply, whereas elaborate marketing, wholesaling and distribution schemes merely increase the cost of food without adding to its quality or quantity. The fourth equilibrium is that of governmental *regulation*. Insufficient standardization and control of food production may permit deception of the public or contribute to actual physical illness, whereas excessive exercise of governmental authority can stifle initiative and delay research and development of new foods and food technology. The fifth equilibrium is that of *education*. Skillful, scientifically based education of the public regarding national food policies and individual food needs, effectively presented by respected authorities, is necessary to counteract perversions of educational processes leading to food faddism or unscientific or uneconomic food practices. The sixth equilibrium is that of *research*. Research motivated by man's drive to regulate his food environment so as to ensure optimum health deserves priority over research designed to gratify minority groups or promote the sales of specific food products.

Considering the magnitude of the food problem and the number of natural and man-made factors entering into the maintenance of even one of these equilibria, the responsibility of each individual with training and experience in the fields of agriculture, food and nutrition, public health, and economics is immediately apparent. No less real is the responsibility of each individual engaged in related fields to survey his own activities and to direct them into channels consistent with achieving and maintaining the previously mentioned equilibria of the food environment. For example, microbiologists have exceptional opportunities to study not only the obvious role of foods as vehicles of disease but also the complex interrelations between bacterial, viral, and parasitic infections and the nutrition of the host. Oncologists have unique possibilities to investigate the deficiencies related to the competition for nutrients between tumor and host in which may lie many keys for unlocking the secrets of cancer. The same responsibility for recognizing, pursuing and reporting on nutrition problems lies with workers in scientific fields not traditionally associated with the nutrition sciences and in the nonscientific areas of agriculture, economics, commerce and politics.

With these six equilibria as goals toward which man's effort is to be directed, it is now important to assess our present positions in relation to these goals.

First, how close to the desired equilibrium are we with respect to *quantity*? A causal inspection suggests that the goal, while distant, is nonetheless attainable. Recent nutrition surveys in widely scattered parts of the world have revealed only moderate per capita caloric deficits but far more serious deficiencies of proteins and certain vitamins, particularly vitamins B_2 and A. The major issue of annual fluctuations in food productions in regions where adequate food supplies are available only in good years has yet to be solved. The emergency redistribution of food surplus to any country experiencing famine is a humanitarian expedient to mitigate suffering, but it is not a long-range solution. Farsighted economists and political leaders have recognized the social and economic dangers inherent to the reliance on emergency importation of food supplies. The ultimate, long-range solution must, therefore, lie in the integrated attacks on the problem through such measures as the development of water resources, improvement in fertilizing practices and the introduction of new crops and new varieties of old crops. Land reform and the introduction of modern agricultural methods, both aimed at increasing and normalizing annual production, de-

serve the attention of individual governments where indicated. Also needed will be the development and construction of storage facilities for the preservation of harvested crop surpluses in good years for use during bad ones.

In present food surplus regions, the maximum skill of the economic planners and political leaders will be required during the next decade to smooth the transition from excess to adequacy of food supply. The gradual decrease of surpluses and the concomitant elimination from world markets of the constant threat to agricultural commodity prices should encourage food production in regions not fully exploited.

Where food is easily available, educational campaigns have stressed the evils of gluttony and are making progress in convincing populations of the virtues of maintaining an ideal rather than an average weight throughout life. With the slow but steady progress toward abundant food for all people, there is and will continue to be a steady increase in the prevalence of obesity and of the numerous chronic illnesses related to overeating as shown by convincing statistical and experimental data. The tragedy of exchanging the human suffering and economic losses associated with undernutrition for the dire consequences of overnutrition can be prevented by a concerted program of research into the pathogenesis of overeating, including in particular the psychological aspects of the problem, and by continuation and extension of present campaigns of mass education.

Second, how close to the desired equilibrium are we with respect to *quality*? The last 15 years have witnessed unparalleled advances in our understanding of the importance of food quality. As one example in the protein field alone, basic research on the biologic value of single proteins, mixtures, and their availability have emphasized the importance of protein quality. This research has led to the recognition of such malnutrition problems as pellagra, among maize-eaters, and kwashiorkor, among widely separated populations who have in common their feeding to young children a dietary which is adequate in calories but low in protein of high biologic value. In Asia, Africa and Central America, knowledge derived from research has been used to formulate, develop, test and actually submit to field acceptability and marketing trials low-cost mixtures of indigenous vegetable proteins. The skillful blending, based on painstaking research, of inexpensive and low-quality protein sources to produce a concentrated protein mixture of high biologic value has been an outstanding development which is accelerating the rate at which protein deficiency diseases are being eliminated from areas of the world where they are now endemic.

Needless to say, desirable improvement in food quality has not been confined to technically underdeveloped areas. The enrichment of white flour with B-complex vitamins has restored to white bread the losses sustained during the milling processes, and the addition of lysin can actually improve the nutritional quality of the original wheat itself. The fortification of margarine with vitamin A has resulted in a product as nutritious as butter, and even more constant in its quality. The introduction of a new process in the production of dry skim milk powder, making the solution of the powder in water almost instantaneous, has led to wider acceptance and higher consumption of milk solids, with resulting improvement of the protein quality in the whole dietary.

Efficient methods for control of insect pests, better transportation of food and food products, sanitary packaging and display of fresh foods and widespread use of screening, insecticides and refrigeration in the homes have led, in more technically developed regions, to greatly heightened nutritional quality of foods. In particular, these advances have determined the virtual elimination of food and food products as vehicles of disease. Even fresh fruits and garden vegetables may now be consumed uncooked and as marketed in many parts of the world, without fear of contracting bacterial or parasitic diseases. Canned or tinned food products may be purchased and consumed without danger of botulinus toxins.

In the field of edible fats, recent epidemiological studies, clinical experiences and laboratory investigations have revealed new and still somewhat controversial theories, not only as to the quantity desirable but also with respect to the quality of the fatty acids present in the ideal dietary. The extensive research in this field promises to introduce concepts of quality to dietary fat comparable to those now applied to dietary protein. The significance of such a development in the prevention of chronic cardiovascular disease and in the possible prevention and therapy of diabetes, diseases of lipid metabolism and of neoplastic diseases need not be emphasized to this audience.

All efforts at increasing food quality, however, have not been based on nutritional needs or economic desirability. Many so-called improvements in food quality have been instituted more to mollify the whims of food faddists or to promote the sales of a specific food product than to improve basic nutritional quality. One need only scan the advertisements in the press and the periodicals of all nations, or listen to or view the broadcasts of commercial radio and television, to become aware of the vast expenditure of time, money, and natural and human resources diverted from scientifically sound, nutritionally constructive and economically sensible efforts at improving the food quality into pseudoscientific and often ludicrous attempts to lure gullible sales prospects into purchasing a product of presumably superior quality.

Third, how close to the desired equilibrium are we with respect to *distribution*? The distribution of foods and food products has changed dramatically with improvements in transportation. The expansion of water and rail communication facilities, the construction of vast networks of roads suitable for motor vehicles and the remarkable development of transport aircraft and the ubiquitous locations of airfields and landing strips have

created a world in which man himself has become the main obstacle to a uniform and constantly adequate distribution of food supplies. Certain temporary seasonal and climatic breakdowns in food distribution systems occur even in the most technically advanced regions; usually they are of short duration and have little nutritional significance. More serious are natural disasters such as earthquakes, floods, fires and storms which disrupt means of transportation over wide areas. In technically underdeveloped regions the effects of such disasters may require longer periods for readjustment.

Man-made obstacles to the adequate distribution of food supplies, however, provide a major source of difficulty. Wars, strikes and civil disturbances continue to disrupt the normal channels of distribution and impose consequent suffering. Lesser man-made problems arise out of ignorance and carelessness. For example, failure to plan for the proper distribution of fruit and vegetables throughout all seasons of the year may result in excesses of dietary vitamins C and A during the height of the picking season in a given region; but when these foods are scarce, the respective vitamin deficiency in the dietary may cause scurvy or xerophthalmia to become a problem. Knowledge that distribution breakdowns are potentially omnipresent, and proper planning to prevent such catastrophies or to cope with them if they occur, is a matter of the utmost priority.

Distribution procedures can, however, become so elaborate as to increase food costs out of proportion to the benefit derived in terms of quantity and quality of food produced. The trend toward more and more intermediaries or middle men between the producers and the consumers has been a major factor in increasing food costs at the retail level. One need only compare the prices earned by the producer with the prices paid by the consumer to obtain a striking example of this essentially unproductive development. The importation of highly processed, costly foods into technically underdeveloped nations under the guise of protecting certain segments of the population from disease is a specific example of an unnecessary food distribution practice. Obviously, food distribution problems are not insoluble. The means for balanced distribution are at hand. By proper planning and by general public refusal to purchase foods containing an unreasonable distribution cost, the gap between present status and the ideal equilibrium with respect to food distribution can be rapidly closed.

Fourth, how close to the desired equilibrium are we with respect to governmental *regulation*? Evaluation of a world-wide position with respect to this goal defies brief summarization because of the variable amount of control ranging from none at all in some nations to total control in others.

With respect to food production, some forms of governmental persuasion to encourage agricultural production in food importing countries, to prevent wide fluctuation in food production in others, and to control excessive production in still others, have been accepted in almost all nations. None have been universally popular and none universally successful. All too often food production policies have been geared to economic policy rather than biologic needs, as for example the production of items like cattle or cotton for export when basic food requirements have not been met. In addition, the rugged individualism of the farmer throughout the world has made regulation of food production difficult. Occasionally, governments have resorted to forceful measures to bring about conformity with programmed production schedules. However, the most powerful government weapons—short of forceful action—have been taxation and credit control. By these devices, in one guise or another, most governmental controls are currently exercised. The ability of a government to tax and to control credit just enough to produce desirable regulation, yet not enough to be punitive or to discourage constructive initiative, is an excellent measure of governmental *savoir faire*.

With respect to standards for foods and food products, grave deficiencies exist in many nations; but the evolution of food standards within the procurement agencies of the armed forces of some nations is a most encouraging development. Standards for food and food products are commonplace in technically developed nations. They assure the consumer that the food he buys is not contaminated by harmful amounts of bacteria, parasites or toxins, is of a certain quality, is honestly weighed and measured and deserves a price consistent with its grade. Unfortunately, the average consumer, even in technically developed nations, is frequently unaware of the existence of, and still less aware of the meaning of, standard grades. This situation pinpoints a specific target for a concentrated program of nutrition education.

Government regulation of food packaging or processing is most advanced in technically developed nations. Regulations enforced by central or provincial governments control such matters as the accuracy of weights and measures, the honesty of branding and labelling, the guarantee of proper fill of containers and protection against the distribution of contaminated or decomposed food. Additional regulations are enforced regarding the safety, when used in food production and packaging, of such items as pesticides, preservatives, antioxidants, enzymes, conditioning agents, gas propellants, emulsifiers, amino acids, vitamins, flavors and coloring agents. By and large, these regulations protect the public's health and guarantee the public fair value for expenditures on food. Occasional excesses by regulatory agencies or those speaking for them have, however, been known to occur. It should be apparent, therefore, that government regulation of food packaging and processing, even in technically developed regions, has yet to reach the desired equilibrium. In the technically underdeveloped nations, regulation of food packaging is a relatively new concept, but occasional examples of highly effective measures protecting the public's health may be noted. For example, in many countries, salt must be properly iodized before being sold. Enforcement

of this regulation will in a few years dramatically reduce the prevalence of endemic goiter.

Government regulation of food distribution has already been discussed. In the United States, the distribution of a food from one state into another introduces the produce into interstate commerce, subjects that food to the full regulatory requirements of the Federal Government and involves federal penalties should any federal regulation be violated. Since federal laws are uniform throughout the nation and more effectively enforced than local or state laws, nationwide distribution of food implies conformity, at least in the United States, with a stringent array of federal protective regulations.

Fifth, how close to the desired equilibrium are we with respect to *education*? Obviously, none of the equilibria mentioned can be obtained without education. Education, therefore, is the cornerstone of this whole discussion of food and the protection of health. Much more, education is required on all aspects of food and nutrition and for all segments of the population before it can truly be said that equilibrium has been attained.

Top priority must be given to the advanced training of carefully selected, devoted groups of individuals who will serve as the nuclei about which other educational programs may develop. These groups should not be composed entirely of so-called nutrition educators but should include physicians, biochemists, physiologists, anthropologists, economists, agronomists, nurses and dietitians, to mention a few of the possibilities.

Foremost among the assignments of these highly trained groups should be the education in nutrition of medical and public health students as well as of practicing physicians and workers active in the public health field. The attempts of physicians and public health workers to instruct their patients and population groups in nutrition are seldom appropriate to the need. The fault lies in the inadequate nutrition instruction offered by most schools of medicine and public health. The erroneous concept, still held by many medical schools that nutrition is dietetics, and hence should not dignify the medical curriculum, must be opposed by every individual and agency represented here today. In schools of public health an equally fallacious concept exists that nutrition is the province of the specialist instead of being a necessary ingredient in the training of every public health worker. Doctors of medicine and public health workers are in the forefront of man's effort to regulate his food environment; consequently, they deserve the highest priority in the distribution of nutrition education resources.

Also among the tasks of nutrition specialists will be the education of nonmedically-trained personnel who contact the public through their positions in departments of public health, education, agriculture, and home economics. Indoctrination of these individuals in the fundamentals of nutrition and in modern techniques of education will prepare them for their responsibility in orienting the general public. In technically underdeveloped regions where governmental departments frequently operate with consultants and advisers, the role of the specialized agencies of the United Nations and the bilateral aid programs—such as those of the International Cooperation Administration and the Interdepartmental Committee on Nutrition for National Defense, which provide personnel and equipment to aid in the task of nutrition education—is apparent. This places on those agencies and programs part of the burden of recruiting and training in nutrition not only highly skilled experts but also auxiliary personnel needed for this type of assignment.

The education process must not be directed solely at the so-called grass roots. It is also the responsibility of the nuclei of nutrition experts to extend their influence over persons responsible for the policies of ministries and departments and, whenever necessary, even over the chiefs of governments. Much time may be saved if highly placed officials can be persuaded to become enthusiastic supporters of the nutrition education programs. Their promulgation of laws and regulations governing food and nutrition practices can greatly accelerate the speed of the education process among the public at large. Their statesmanship in accepting and granting to long-range regional and world-wide considerations regarding food and its relation to health, precedence over nationalistic pride and domestic political pressures, in itself can be an educational factor of major significance.

It should be emphasized that nutrition educators are in intense competition for the minds of men in both technically underdeveloped and developed regions. The competition may be with ingrained ignorance and superstition in some areas and with profit-inspired commercial television in others. Nonetheless, the competition is keen, and the antagonists resourceful. The nutrition educator cannot, therefore, live in an ivory tower. He must cope with the realities of his environment, but in so doing he must always bear in mind his ultimate goal and the principles on which his training has been based.

Lastly, how close to the desired equilibrium are we with respect to *research*? Fortunately, this picture is more favorable. The value of research in basic processes of nutrition has been accepted by the management of many food industries and by the heads of governmentally sponsored research institutes, private philanthropies and international agencies. Unfortunately, however, considerable effort is devoted to research only for the purposes of sales promotion.

Perhaps the most needed change in current research activities in the field of nutrition is a far greater emphasis on studies in man in his own environment. This may be accomplished both by direct support of specific programs in human nutrition and by the education of clinical and field investigators and public health workers in other specialized fields to build nutrition research into their own experimental designs. The importance of bringing a vast array of basic and animal investigation to the human level cannot be overemphasized as a

means of accelerating improvement in the nutrition of man and the populations.

It is my hope that this discussion has shed additional light on the key role of education in every effort in nutrition and food science. The attainment of each equilibrium is possible only when progress in education matches progress in other fields.

This summary of our goals and our present position with respect to them suggest that we should have high hopes. Efforts to date have been rewarded by tangible progress in many areas. Great challenges still lie ahead.

In conclusion, let me point out again that man now has the means to reach the desirable equilibria with respect to his food environment. Let us hope that he will strive wisely and consistently towards this purpose which is inherent to his own nature. "Man is," in the words of Albert Camus, "the only creature who refuses to be what he is."

Program of the World Health Organization and plans for the future

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IT IS A GREAT PLEASURE AND PRIVILEGE for me to be able to attend this Fifth International Congress on Nutrition. As you will see from what I have to say about the World Health Organization, nutrition occupies an essential place in our activities and I am therefore looking forward to the conclusions which are to result from the discussions of your Congress. Many of the people participating in these meetings have in the past given very valuable assistance to our Organization and I should like to take this opportunity to extend to them WHO's thanks. There is no doubt that one of the essential conditions for the success of the long-term work WHO is engaged in is action by the greatest possible number of specialists in the various fields of medicine and public health. I must say at the outset that as far as nutrition is concerned we have been particularly fortunate from this point of view and I am convinced that the cooperation between WHO and nutrition experts of all countries will continue to develop in the years to come.

I have been asked to speak about 'the programme of the World Health Organization and plans for the future.' Before discussing the place and significance of nutrition in this programme, which is of particular interest to your Congress, I am sure that you would want me to say a few words about WHO in general and about some of the fundamental principles which have guided its work since its inception some thirteen years ago.

WHO is one of the specialized agencies of the United Nations and the basic idea behind it is the same as that which underlies the whole UN system. It expresses the growing conviction of people everywhere that it is possible today to create a world society in which opportunities for higher standards of living, education and health are available to all people of every colour, race and country.

You should not think of WHO as a supra-national administration, as a kind of supergovernment. We make no laws. We do establish international health regulations, but they are adopted and enforced by the governments themselves. Above all, WHO serves as a great international channel of communication, a sort of world clearing house of information on which all countries can call for help in dealing with their own particular problems.

Indeed, WHO is essentially a catalytic agent. We stimulate and assist national health services in planning and developing their own health programmes. Our technical advisers work with the governments on their request. We also make available teams of field workers to help governments get their new programmes under way. These international personnel are replaced by nationals as rapidly as the latter can be sufficiently trained to carry on the work alone.

While the basic aim of WHO has always been the strengthening of public health services, the methods and techniques for reaching this aim differ widely from one country to another. We touch here another fundamental principle of WHO. It is indeed the Organization's constant concern to avoid the imposition on any country of forms of organization in health services which would not fit that country's cultural and social pattern, or which would be out of line with the development of its economic potential.

Much of the success achieved by WHO in past years is due to its regionalized structure which is eminently suited to meet adequately the health needs of the different parts of the world. Today, regional offices in Brazzaville, Washington, Alexandria, Copenhagen, New Delhi and Manila serve the six geographical areas into which the world was divided in 1948: Africa, the Americas, the Eastern Mediterranean, Europe, South-East Asia and the Western Pacific. One of the essential activities that WHO is carrying out through these six regional offices is to provide assistance to countries in their fight against communicable diseases which, despite considerable progress, still represent major public health problems in the world as a whole. Here the two important developments in the last few years are: the more generalized use of eradication as compared with control techniques, and the shift in importance in some countries from parasitic and bacterial diseases to virus diseases.

The best example of the world-wide acceptance of the concept of eradication as an approach to the problem of communicable diseases is provided by the case of malaria. Since 1955 WHO has been engaged in an all-out attack on this disease and the aim is nothing less than the complete elimination of malaria as a public health problem. You may be interested in a few figures showing the progress we have already made towards the achievement of this goal. By the end of 1959 out of a total of one thousand four hundred million people either affected by malaria or living in malarious areas, close to two

hundred and eighty million live in territories from which malaria has been eliminated and therefore are threatened by this disease only as an outside danger. Sixty-two countries or territories, representing a population of six hundred and eighty-four million, are fully engaged in eradication work.

Smallpox is another target of WHO's eradication policy. By 1961 smallpox eradication campaigns will be in their final stage in some of the countries of the Americas and well advanced in others. In the Eastern Mediterranean, South-East Asia, Western Pacific and African Regions, some countries where the disease still constitutes a serious public health problem will be developing smallpox eradication campaigns or will be completing the planning stage.

For other communicable diseases we shall have to satisfy ourselves with speeding up and improving the control operations which have been going on now for more than twelve years. I am thinking of the fight against tuberculosis, the treponematoses, in particular yaws, trachoma, bilharziasis, leprosy, to mention only a few of the great public health hazards which continue to cause widespread ill health and a very great number of deaths.

I do not wish to convey the impression that we have in fact made great advances in the control of all important communicable diseases. The means of control of some of the diseases most closely associated with malnutrition—the infectious diarrhoeal diseases of childhood and intestinal helminthiasis—has not changed very much in recent years.

The main reason for viewing with optimism the outcome of measures taken against many communicable diseases is that there is growing realization everywhere that any lasting improvement in the health conditions of any given country depends primarily on the establishment of a solid groundwork for its health services. Indeed, while in the first years of WHO's life an over-overwhelming number of requests from countries were for direct assistance in the fight against disease, the great majority of programmes carried out with WHO's help today are long-term projects aimed at the creation or the strengthening of national health services.

Let me give you a few examples of the activities promoted by WHO in order to help countries overcome the lack of adequately-trained health personnel, undoubtedly the most serious single handicap to the development of national health services. We are assisting in the development of training facilities by providing countries with teachers and advisers specialized in particular subjects such as nursing, environmental sanitation, social and occupational health and nutrition. The main duty of these experts is to prepare local staff to take on the teaching functions when the international personnel leaves. In 1959, for example, a total of one thousand, one hundred and fifty-eight months were served by one hundred and twenty-nine teachers in nineteen disciplines in twenty-seven countries. Another method of improving education and training of medical and allied personnel has been to provide opportunities for studies and observation abroad. Since 1947 more than ten thousand fellowships have been awarded for such a purpose and the total for 1959 was one thousand, four hundred and thirty-one. Of these 56% went to physicians, 12% to nurses and midwives, 7% to sanitary engineers and 25% to other health personnel. The truly international character of WHO's fellowship system is shown by the fact that fellows from one hundred and twelve countries went to eighty-nine countries, the majority of them to the UK, USA, France, Denmark, Sweden, India, the Netherlands and the United Arab Republic.

WHO's task of promoting training and education of health personnel, as well as of channelling to all countries knowledge and experience acquired in the fields of medicine and public health, is greatly facilitated by the system of expert advisory panels which provide the Organization with information and guidance for all the programmes it is engaged in. These panels—the number of which has now grown to thirty-eight—comprise large numbers of the world's leading medical scientists and health administrators in the fields of work in which WHO is interested. The views and recommendations expressed by panel members, individually or collectively in the form of expert committees, help in formulating general policies or establishing concrete programmes for future work.

Mention must be made of two activities of WHO of direct importance to you. The first refers to the effort the Organization is making to promote an adequate and uniform system of recording vital and health statistics in many parts of the world. Unless this is done we shall not be able to measure the magnitude of such essential problems as, for instance, the extent to which the combination of infection and malnutrition is responsible for mortality and morbidity, particularly in early childhood.

Health education of the public is another branch of WHO's activities which has an influence on the development of nutrition programmes. In order to enroll the active participation of the public, without which these programmes cannot be developed, we must try to understand the attitudes of the people towards change and the social and economic barriers which oppose such change. The collaboration of health educators, anthropologists, psychologists and representatives of other disciplines indispensable to such an undertaking, is exemplified by the conference on changing food habits which is to take place in Cuernavaca in Mexico next week. I am sure that this conference, sponsored by the World Federation for Mental Health and by the Josiah Macy, Jr. Foundation with the participation of the representatives of various international agencies, will yield important results for the nutrition programmes of each organization.

In relating nutrition to other activities of WHO I have so far given examples of primary importance to the economically under-developed countries. Nutrition however plays an important role in many programmes

carried out by WHO which are of immediate concern to the so-called developed nations. Let me give you one instance only. In the last few years WHO has been actively sponsoring the study of the part nutrition plays in cardiovascular diseases and in particular in the etiology of ischaemic heart disease.

It is time now for me to describe briefly the evolution of the nutrition work WHO has developed in the last twelve years.

As early as 1949 it was decided, after a review of nutritional diseases carried out jointly by FAO and WHO, that priority must be given to the question of protein malnutrition in the early years of life following weaning. Since then we have helped many countries to carry out surveys with a view to establishing the prevalence of this condition, the etiological factors responsible, and the morbidity and mortality caused by it. We have also organized world-wide and regional meetings in order to stimulate and co-ordinate research on this problem. I should like to pay tribute here to the Josiah Macy, Jr. Foundation, which I have already mentioned, for the Foundation has enabled us to convene two very productive meetings of research workers, one in 1953 and one in 1955. Other forms of assistance provided by WHO in this field were the exchange of workers engaged in research in India, Africa, and Central America, and the provision of grants to the centres engaged in research on the development of protein-rich food. Finally, let me mention the protein advisory group which gives valuable guidance to the Organization, to FAO and to UNICEF in their respective fields of work.

Another programme which we are carrying out in cooperation with FAO is related to food additives. This work was started in 1955, on the recommendation of a joint FAO/WHO Committee on Nutrition. During the intervening years a number of expert committee meetings dealing with the general principles governing the use, procedures of testing, and specifications of food additives have been convened and their reports have been issued. A study group was also convened to advise WHO on the need for further research on the carcinogenicity of food additives.

In the beginning of our programme in nutrition, a great deal of attention was paid to endemic goitre. This condition, as you know, has been successfully prevented in Switzerland and in other countries by the enrichment of the refined salt which is in general use, with potassium iodide. In countries where only crude salt is used this method proved not to be applicable and this appeared to be a problem which could well receive attention from an international organization. Some surveys of disease incidence, and of the means of control that were used or had been attempted, were carried out. It appeared from these that if a satisfactory method of enrichment of crude salt with iodine could be developed this was likely to be most effective. The matter was then taken up with the Chilean Iodine Educational Bureau and investigations were begun into the stability of iodine preparation in different conditions of temperature, moisture and impurities of salt. The best results were attained with iodates. The absence of toxicity of iodates and availability of the iodine to the thyroid gland from iodates ingested orally were demonstrated by enquiries carried out at WHO's suggestion by the Medical Research Council of the United Kingdom and the Food and Drugs Administration of the United States Public Health Laboratories. Lastly, the effectiveness of iodates in the prevention of goitre was adequately demonstrated by the Institute of Nutrition of Central America and Panama in well-controlled field experiments carried out in Central America.

More recently the Organization has turned its attention to anaemia which, of course, is widespread in many of the less developed countries and is responsible for much mortality, morbidity and economic loss. From the reports of the many consultants who helped WHO on this problem, and according to the findings of study groups which reviewed the whole field, it appears that this is not a simple nutritional problem and that again the question of the association of other diseases will have to be considered. In view of the apparent differences in the prevalence of this disease and possbliy its nature in different circumstances, it would seem that it is essentially a suitable subject for study at the international level. Such a study might well indicate to us how practicable, preventive measures could be developed.

Another important field of study is the nutritional diseases of the eye. The most important of these is, of course, hypovitaminosis A which, surprisingly enough, is most prevalent in countries in the tropical rain belt—where the precursor of vitamin A—carotene—is most plentiful. In one area, between 1 and 3% of pre-school age children are said to suffer from an acute deficiency resulting not only in much blindness but also in high mortality. The association of the condition with protein malnutrition and with heavy intestinal parasitism has been studied in conjunction with WHO field teams and consultants. Plans for further investigations have now been drawn up and it is hoped to carry out further enquiries which will lead to the development of means of prevention.

There is another aspect of nutrition in relation to diseases of the eye which is of particular interest to WHO at the present time. Trachoma is still a widespread disease and large-scale control measures have been developed with WHO assistance. Surveys have revealed that there are great differences in the severity of this condition and the associated infections, even within communities in close proximity to each other. There are therefore environmental influences other than the infection which account for these differences and the nature of these is now under study. Epidemiological studies now under way will be considerably developed in the future and these will include enquiries into the part that nutrition may play in deciding both the incidence and severity of the disease.

Beriberi appears still to be an important public health

hazard in certain countries because in rural areas where rice was previously milled by the primitive age-old hand pounder, power-driven mills are now being introduced. It is not practicable to attempt to delay this change and other methods of evolving an adequate intake of vitamin B must be found. Meantime, we are not informed on the magnitude of the problem. There is evidence that it causes a high infantile death rate. WHO has already carried out some investigation but further work is needed. The same applies to pellagra which is of special importance to the continent of Africa.

In outlining the various activities of WHO, I have several times referred to the need to undertake extensive research to obtain facts and knowledge indispensable to the prevention and control of most of the diseases. The constitutional mandate given to WHO included the stimulation and coordination of medical research; but because of restrictions in manpower and in financial means, this aspect of WHO's work was developed only to a very limited extent during the first decade of the Organization's life. By the end of that period, however, there were clear indications that greater progress in world health would depend to a very significant degree on the ability of WHO to take maximum advantage of the resources which are available in the world for the promotion of medical research. It was on the initiative of the Delegation of the United States of America that the Eleventh World Health Assembly two years ago decided that in the years to come WHO should take the responsibility of establishing an intensified international medical research programme. Let me say at the outset, that we continue to believe that research is and should remain a predominantly national responsibility and that it is the individual research worker who is at the heart of every scientific investigation. It follows that successful research depends upon maximum freedom for the individual to develop his own potential and his own ideas. As in the past, therefore, WHO will refrain from undertaking research on its own. Our primary purpose is to bring together the various research activities going on in many countries in order to avoid unnecessary duplication and overlapping and to define clearly the fields in which international research is needed. Such coordination, which has always been necessary, has today become even more important because of the accelerated speed and large scope of research.

As far as nutrition is concerned, we have so far been able to establish communications among research workers in different parts of the world and as a result of this it has been possible to see the neglected areas where investigation is needed and recognize centres competent to carry out research but restrained from doing so by lack of manpower or money or both. As to the type of research which in nutrition would require international organization, let me just cite the need for measuring the incidence and prevalence of different nutritional diseases under varying dietary and other influences, the need for an epidemiological and interdisciplinary approach to nutritional problems and for base-line studies in growth and development.

We are of course still at an early stage of our research programme. A document outlining the main fields in which research should be undertaken was drawn up by consultants and reviewed early this year by a scientific group. Every effort will be made to find the resources that are required for expanding this work. Research in nutrition is likely to become increasingly important as other causes of ill health come under control, and I can assure you that this is clearly recognized by WHO.

I have endeavoured to give you a picture of the work of WHO as it is directly and indirectly related to the problems of nutritional diseases and to indicate to you what our plans are for the future. However, as I said at the beginning of my remarks, in nutrition, as in other branches of our activity, we can hope to go forward only if we are assured of the active participation in our programmes of all medical workers and scientists in a growing number of countries. I am convinced that, thanks to your cooperation, the improvement of nutrition will lead to the betterment of health conditions all over the world, a goal which your Congress shares with our Organization.

Banquet and Closing Session Addresses

The world's children and tomorrow

MAURICE PATE

United Nations Children Fund, New York City

MAY I TELL YOU how pleased I am to participate in this your Fifth Congress and how grateful I am for this opportunity of discussing with you some of our mutual interests. I have visited the countries of most of the delegates to the Congress in the economically underdeveloped regions of the world, and have observed the problems you have in the area of child welfare. I have seen the encouraging results of your diligent and persevering work.

The United Nations Children's Fund, known by the letters UNICEF, is a fund set up by resolution of the General Assembly of the United Nations to benefit children in the economically underdeveloped countries. It does this mostly by making grants in aid to programs benefiting children in some one hundred countries. The Fund received last year the equivalent of $24 million, which is of course a very small sum in relation to the general purposes of UNICEF. This assistance is allocated to projects by the 30-nation Board that governs the Fund, and is usually delivered in the form of imported supplies required by the project. The greater part of the cost of projects is furnished by the countries that ask and receive UNICEF help, for they provide local financing, personnel, and facilities, and they operate the programs. The total cost of projects to which grants were made by UNICEF during the 14 years of its existence comes to the equivalent of about one billion dollars, two thirds of which has been paid by the beneficiary countries out of their own modest resources. At the same time the thanks of the children of these countries go out to some ninety governments contributing voluntarily to this work, to a large number of nongovernmental organizations, and to millions of individuals contributing to UNICEF.

How is UNICEF related in the UN family, to FAO and WHO and the U.N. Technical Assistance Programs UNICEF is a fund making grants to country programs and *not* a technical organization. Countries asking grant? from UNICEF generally also request technical assistance from FAO or WHO or other U.N. bodies for the same project. Our Board has the benefit of the technical advice of FAO and WHO when considering its policy, and the best ways to use its resources to improve the conditions of children. According to an agreed division of responsibility our staff does not duplicate the technical services available from FAO and WHO.

There are some 600 million children in developing countries where the annual income per inhabitant is under $100. From the vast needs of this group, our Board has concentrated its assistance on projects for children in the field of health, nutrition and, just recently, social welfare. Only a small part of these fields can be assisted with UNICEF's modest resources. In the health field, for example, UNICEF gives assistance for extending rural health services, for public health campaigns against major diseases affecting children, such as malaria, tuberculosis, yaws, trachoma, and leprosy, and for training local personnel.

When we in UNICEF look at the needs of children in the developing countries, hunger and malnutrition are certainly in the highest priority. When you in this Congress look at the problem of hunger and malnutrition in the world, you certainly see that they bear most heavily on the young children.

If a rough division is made of projects assisted by UNICEF into projects for health and projects for nutrition, you find that ¾ of UNICEF's resources have gone to health projects, and ¼ to nutrition. This means that governments have sought much more help for child health than for child nutrition.

I believe that among the reasons for this are: the cost and difficulty of improving nutrition; the less advanced state of government planning; the absence of a food policy linking agriculture and nutrition; lack of trained personnel; and lack of experience in the field of applied nutrition. This Congress and the work it inspires will certainly contribute to the reduction of these obstacles.

UNICEF's assistance to the improvement of nutrition began with emergency milk distribution in the war-devastated countries. In fact, the symbol of UNICEF was a cup of milk, and an Italian child once explained "UNICEF means cow!" The distribution of milk continues even now in about the same quantity—the half million metric tons moved by UNICEF in the last 14 years would make a trainload extending from Baltimore to New York. The program has changed greatly. The milk now comes as a special donation from countries with a surplus—United States of America, Canada, and most recently, Switzerland. UNICEF pays only the overseas freight which, however, absorbs more than 10% of our annual budget. The milk now goes only to the economically underdeveloped countries, and more than half of it goes to the vulnerable pre-school age group. The most important change is an attempt, in which

FAO and WHO are highly interested, to have nutrition education carried with the milk distribution program. We can see in many regions a remarkable increase in the demand for milk and eventually other protective foods.

Our next effort was to help countries start industrial processing of milk to increase their supply for urban areas, knowing that in most areas of low milk supply a greater part is given to children. At the same time FAO helps countries to improve milk production and quality in the areas concerned, and to develop a general milk policy. UNICEF has provided milk processing equipment for 154 fluid milk plants and 32 milk drying plants in 31 countries. These plants include provision for the marketing of part of the output in the form of low-fat milk to help meet the difficulty of the high cost of milk in relation to the income level of the developing countries. Probably the city of Bombay has gone furthest in this way by selling a litre of low-fat milk for 5¢.

The widespread recognition of protein deficiency in pre-school children in tropical countries, and the relatively high cost of milk in these countries, led to a program sponsored by the international agencies and several foundations to develop inexpensive protein foods for children. Up to the present, 30 research groups located in 16 countries have participated in this development with the assistance of two generous grants from the Rockefeller Foundation. Since this program is, I believe, known to many members of the Congress, I will only express my thanks to those who are giving the benefit of their time and experience to both the planning and the execution of this work.

A number of products that appear suitable have already been developed, and the next stage is to encourage large-scale production and consumption in the areas where they are needed. UNICEF has been aiding in the process development and acceptability tests. Indonesia is producing a product called saridele processed from soya beans and sesame. A plant for making fish flour will be ready for operation in Chile later this year. Two peanut flour plants will begin operation in India next year. In this stage of the program, we hope to have the cooperation of business enterprise.

However, the majority of children in the developing countries are in rural areas which are as yet more in a subsistence than a money economy. There is a vast need for the improvement of community nutrition by more production and consumption of protective foods and especially the traditional protein-rich foods such as pulses, eggs, milk, fish and meat. It seems that community development, agricultural extension, schools and health centers could be used more to give nutrition education, and to expand the right kind of food production in the community. Since 1957 trials are being made in a few countries in each continent with the help of the international agencies.

I have tried to give you a glimpse of what we are doing to improve child nutrition. Certainly it is apparent wherever I go that malnutrition is the greatest obstacle to the well-being of children. In the fields of health and nutrition we seek the help of technical people, like yourselves, to help define the ways in which UNICEF can be more effective in its work. Our UNICEF Board is increasingly concerned with the nutritional needs of children and we are therefore seeking guidance as to how we can use the technical resources and the available foods in the world to meet the general problem.

Since the resources contributed voluntarily each year have tended to rise from year to year, we expect the amount of UNICEF funds going into nutrition to increase, while fully recognizing that they will represent only a minor part of what is needed in this field. Our Board has decided to give its fullest support during the next five years to the Freedom from Hunger Campaign sponsored by FAO.

Mr. President, I would like to say that the Nutrition Fraternity is one of the greatest brotherhoods with which I have ever had the privilege to work. Everyone helps each other in a remarkable way and thus your all too few hands in this field have become a great power for good.

My first contact with nutrition was 40-odd years ago when Dr. Alonzo Taylor was Mr. Herbert Hoover's nutrition Adviser in prescribing the proteins and other valuable foods that went into a nourishing daily supplementary meal to 16,000,000 children in European countries and Russia after World War I. Dr. Taylor's knowledge, combined with an exuberant and convincing personality, was quite an eye-opener for me as a young man. And of course nutrition has gone a long way since and my country has learned much from scientists of other countries.

I am sure that you who have come here from all over the world will return to your posts of duty with new inspiration. And for those of you who represent countries in process of development I want you to know that UNICEF, in concert with our colleagues in FAO and WHO, is prepared to give you all the backing it possibly can towards carrying out your very worthy objectives.

A 'five-point program' for industry's role in improving global nutrition

H. J. HEINZ II
H. J. Heinz Company, Pittsburgh, Pennsylvania

SOME TIME AGO, when Dr. King invited me to speak at this dinner tonight, I felt not only greatly honored, but I accepted the invitation with a genuine feeling of pleasure, because the subject of food and nutrition has been close to my family for many years. It has also been a rewarding experience to be part of a great industry—one that links agriculture, food technology and nutrition. Furthermore, many of us have worldwide interest of many years standing, so it is only natural that our industry should be especially interested in this Conference.

Since food is the basis, the very sustenance of living, no one can talk about the food industry without considering the broader aspects of man's continuing struggle to survive.

May I, therefore, talk to you tonight about mankind's long struggle to master his environment. There have been many conquests, many triumphs. Diseases that have scourged and plagued mankind for centuries have been conquered. Traveling around the globe in 48 hours has become commonplace. Our voice and image can be transmitted mechanically not only to any part of this earth, but also to outer space in a matter of seconds. Man-made satellites are circling the globe like so many moons; and the proverbial trip to the moon is almost within our grasp. Man and science are racing to the furthest reaches of space to stagger the imagination.

Our thoughts are becoming more and more preoccupied with what we will find and do on other planets, but right here on this planet, right here and now, a basic problem, the most fundamental one of all, continues to haunt us. Man is still suffering and, in some places, even dying of hunger. The dread twin spectres of hunger and serious malnutrition constantly stalk over half the population on this planet. Food is the staff of life. Nevertheless, it is estimated that more than a billion people in the underdeveloped countries of the world are not getting enough food for proper physical development, for adequate resistance to disease, for the day-to-day requirement of living.

The problem is therefore self-evident. In simple terms, it is to extend the techniques which have brought freedom from want to the few—to all of mankind.

Much has been accomplished, much more remains to be accomplished.

What has been the food industry's contribution?

Within the short span of one generation, there has been a literal revolution in food technology. May I offer some concrete illustrations:

In America, with the help of applied research, it has provided in many areas a twelve-month market for a one-month crop. Food technology has now made it possible to deliver an extraordinary variety of inexpensive and nutritious processed foods almost anywhere in the world.

Of outstanding significance are the more-than 125 different fruits, vegetables, or meat-and-vegetable combinations for infants and juniors, of special nutritive formulation and texture, convenient and highly palatable to adults as well. Dietetic foods, too, have been developed for special needs.

Processed citrus products, high in vitamin C, are now abundant in areas where they were either nonexistent or priced out of reach.

Through one of the century's major accomplishments in food technology, dehydrated instant milk is now a reality.

Through new techniques in packaging, shipping, handling, and refrigeration, it is now possible to protect fresh as well as processed foods and give them longer life. Precooling, for example, greatly lowers spoilage risks in handling milk, poultry and newly-slaughtered beef. Cellophane packaging of meat in retail quantities is now common practice, and already about one-fourth of all fruits and vegetables is pre-packaged before reaching retail levels. Thus moisture loss is retarded, keeping the products fresh and attractive.

The number of items on the shelves of the large supermarket has grown from about 4,000 in 1946 to 8,000 today. At least two-thirds of these are new and improved items. The vast majority are processed in one form or another. They are canned, waxed and dried. They are frozen, bottled and pickled. They are wrapped, packaged, and baked. One way or another, the food industry stabilizes its products.

These 'convenience' products often save the consumer money. For instance, a pound of shelled peas, frozen or canned, costs about 32 cents. Bought fresh, the same quantity of peas costs about 70 cents. Citrus juices made

from frozen concentrate cost about half as much as the same amount squeezed from fresh fruits.

In assessing the contribution of our industry, applied research has resulted in making available to the housewife a vast variety of better, more uniform products, often at less cost, and without sacrifice of nutritive quality.

A bottle of ketchup today costs the same at retail as it did in 1923. Let us, however, not forget that while these cost reductions represent great advantages in food processing technology, millions of people throughout the world cannot even begin to afford such 'convenience' products.

The food industry is the world's largest, with total consumer spending of about $76 billion for food in 1960. These food dollars employ directly and indirectly about one-quarter of the world's working population—in agriculture, in food manufacturing, transportation, distribution and supplier industries.

These are some of the remarkable advances we have seen, but the food industry has actually only started its forward march. Continuing research is pointing the way.

We fully realize that fundamental as well as applied research is an essential ingredient in the growth of our food technology. Today, the research scientist has come into his own. He works in specially designed centers, has costly apparatus at his elbow, and he is an important member of industry. The food manufacturers of the world, with their many-faceted technological programs, are helping to upgrade the nutritional levels of their own populations, of others as well. The American food industry annually invests a total of $100,000,000 in research. By 1965, it is expected that this amount will increase by 40%, a rise far greater than the average for manufacturing industries. In addition, $400,000,000 is being spent by national and state governments in this country on farm research. This is double the figure expended only ten years ago.

We, of the food processing industry, will continue to support vigorously sound research in the nutritional sciences.

Work is proceeding at a stepped-up rate in industry centers, university laboratories and through unique organizations such as the Nutrition Foundation.

This scientific research organization is totally supported by the United States and Canadian food and related industries. The Foundation was organized as a sincere expression of their interest in scientific progress and the health of humanity. Starting with 15 members 19 years ago, Foundation membership has now grown to nearly 60 companies. The Foundation provides grants to universities and medical schools in support of basic nutrition studies. While the funds originate from contributions made by the food industry, the assignment to specific research projects is under the full and independent control of a special scientific advisory committee to which the trustees of the Foundation give only final ratification.

Over the years, many millions of dollars in grants have been made to university and medical centers in the United States, Canada, and Central America. These projects have contributed enormously to our knowledge. The recipients of Foundation grants have made—and are continuing to make—significant contributions to the science of nutrition with respect to proteins, fats, carbohydrates, minerals, vitamins, infant and maternal nutrition, dental caries, anemia, diabetes and nutritive factors affecting heart and vascular disease. May I cite just two examples: A Foundation grant extending over ten years made possible the classic work of Dr. W. C. Rose at the University of Illinois in identifying the amino acids that proteins must furnish to protect health in young adults.

The Foundation was further privileged to assist the work of the Institute of Nutrition of Central America and Panama, in Guatamala City—the well-known INCAP. It helped INCAP to formulate a cheap dietary supplement for the undernourished people of the area. The new food—called 'Incaparina'—is high in protein, essential minerals and vitamins. It is made from a blend of locally available foods, including corn, sorghum and cotton-seed flour, plus yeast and vitamin A. The Central Americans have found it to be tasty; what's more, it is cheap, costing only 3¢ for a day's ration.

We of the food industry pledge that with the passage of time the Foundation will continue to grow and that the scope of its work will broaden even further. In addition, we will, of course, continue to work closely with and through such organizations as FAO, WHO, UNICEF, Pan American Health Organization (PAHO), ICA and others, dedicated to the betterment of nutritional practices throughout the world.

In 1954, the 83rd Congress of the United States passed the Agricultural Trade Development and Assistance Act, better known as Public Law 480. Time and changing conditions brought a change of sentiment as expressed in the 1959 Amendment, with which I heartily agree. This amendment is known as 'The International Food for Peace Act of 1959.' In it, the Congress declares, and I quote: "In view of the abundant agricultural production of the American farmer made possible by the advances of science and technology, and the continued hunger and want of clothing in many areas of the world, the Congress declares it to be the policy of the United States, in cooperation with other friendly nations, to put its abundance of supplies, as effectively and rapidly as possible in the service of human need. The Congress further declares it to be the policy of the United States to utilize our growing stocks of agricultural goods to aid in all possible ways those peoples who are in revolt against poverty, illiteracy, and disease, and who are determined to achieve that economic and social development necessary to national dignity and individual well being."

We, in the food industry, pledge our support to the fruition of the noble purposes and lofty ideals of this Act. Even under the original act, out of $27 billion in agricultural products that were exported by this coun-

try, more than $8 billion were dispatched to friendly peoples under special 'humanitarian' programs.

Under all aid programs the United States has sent nearly 6,500 shiploads of farm products to more than one hundred foreign countries. Such programs are designed to meet the needs of these countries within limits under which they can be physically transported and distributed. These projects must and will continue to grow in scope and depth at an ever increasing pace. In this effort *we urge the increased cooperation of other favored nations.*

If I may, I should now like to turn to the challenges of the future. *We are now faced with a population explosion!* Modern medical science and technology, with their triumphs in death control, have created a problem that staggers the imagination. Since the world population is expanding by more than 50 million people annually, it will take only 35 years to double our numbers. If today we are having trouble in producing adequate supplies of food for the present world population of 3,000 million people, what will be the food problems of 1995?

Certainly, if civilization on this earth is to continue, international political tensions and disagreements must be resolved. But the problem of population and food supplies cuts across all political boundaries and must also be solved.

Food production, in its race with the population growth, has barely managed to hold its own—2% against 1.6% on the world average. Unfortunately, the most spectacular increases in food productions have been made mostly in countries which already have an abundance of food. Yet, large areas in Asia, Africa and Latin America are falling behind in the race and have actually lost ground. This is true in spite of much good work by government and technical assistance agencies of United Nations. I emphasize the fact because I sincerely believe we must intensify our efforts and do some new things as well.

The experience of industrially and agriculturally advanced countries shows that, by the application of scientific methods and proper management of the economy, productivity of agriculture can be increased to limits hitherto believed unattainable. In the United States, the phenomenal success of intensive cultivation seems seriously to challenge the validity of diminishing returns. Improved agricultural tools and machinery, rational use of fertilizers, research in genetics and in pest control, farm credit and marketing arrangements can bring about a radical change in the present low-yielding agricultural practices of many countries. It is the duty, indeed the obligation, of the more industrially advanced countries to help.

I submit to you that this is the great problem and challenge of our time. I know I can speak for my colleagues in the food industries throughout the United States when I say we will play our part and we will welcome the challenge. Furthermore, I believe that the food industry throughout the world feels this way.

How can we do our part?

As I see it, the problem must be attacked boldly on a broad front. The food industry knows full well that foods and nutrition are interrelated concepts which cannot be divorced and that it is up to industry to shoulder an ever-increasing responsibility in the field of nutrition. This responsibility can be expressed in manifold practical ways. I, therefore, submit to you a five point program.

Point one: There is a serious shortage in the underdeveloped countries of trained personnel with sufficient basic experience in the science of nutrition to enable them to explore and identify the nature of the nutrition problems of their own country and to direct their solution. To meet this need, I propose that there be established nutrition scholarships for the countries in greatest need, to be financed by the food industry and government, as well as by interested foundations and world organizations—to train students in nutritional sciences, in nutrition education, and in related fields. I suggest that the criteria of selection of such individuals be set at practical levels, recognizing the educational progress and the kind of problems existing in each country. I would think that a qualified committee of men like Dr. Sen, Dr. King and others who guided this Congress might be asked to determine the feasibility of this project, the necessary criteria, and the kind of study that would be useful. Furthermore, I would like to say that in order to get this program going, the H. J. Heinz Company will offer the first three such scholarships to qualified individuals, which will include transportation, tuition, and living expenses.

Point two: There is an increasing recognition of the importance of nutrition for the maintenance of health. Nevertheless, too few medical schools devote more than a token minimum of time to the teaching of normal and clinical nutrition to physicians and others active in protecting public health. I propose that it would be a step in the right direction if the food industry, either acting as individual units or in concert, would endow chairs of nutrition in interested medical schools. Similar concrete encouragement should be offered to selected university graduate schools and schools of public health—to facilitate the research and teaching in the science of nutrition and to extend to the specialists in nutrition the support and stature they deserve.

Point three: At a time of increasing public awareness of the relationships between nutrition and health, there exist few sources of reliable information to the layman. In default, the field is exploited by assorted charlatans who rush in to capitalize on public ignorance and anxiety. Thus the public is at the receiving end of a wide spectrum of very dubious nutrition information. True—an increasing effort for better nutrition education is made by Government agencies, public health departments and schools, but I feel that additional education is needed to reach larger segments of the population.

I propose, therefore, that the food industry at large should sponsor a nutrition education program, possibly within the framework of the Nutrition Foundation,

whose aim would be the dissemination through all mass media of communication, authoritative, reliable, nutritional information. Such an organization, cooperating seriously will all agencies conducting nutrition research, could contribute greatly to public nutrition education and to improved eating habits.

Point four: I propose that our food and related industries seek the cooperation of comparable companies in other countries to support the publication of a journal in each major language, covering research advances as now accomplished by NUTRITION REVIEWS, in the belief that this will add greatly to the applications of scientific knowledge in fields related closely to nutrition.

Point five: There is a real opportunity to share more fully our technical progress with the people of underfed countries to improve their food and nutrition practices, in parallel with our own progress and their's. The long-range solution lies in helping the underfed countries to produce and process ample food *for themselves.* We of the food industry who are presently fortunate, have a special responsibility to share in work all over the world in preparing more food and better food. In the field of agriculture, the food processor can and does supplement and cooperate with the scientific agriculturist. For example, my own company is working to improve the yield per acre and the quality of the tomato crop, to lower the cost of tomatoes in Italy. During the past four years, our senior agronomist and his staff have been advising with Italian farmers on the adoption and adaptation of tomato culture practices that have been productive in this country. In the State of Ohio for instance, crop protection techniques (e.g. anti-blight spraying) have doubled the yield per acre of Heinz contract farmers in the last ten years.

Many of you are familiar, I am sure, with the remarkable accomplishments of the Rockefeller Foundation, working in cooperation with Mexico, in doubling their corn production within about 20 years.

Back in 1935, I journeyed to Australia, purchased a local piano factory that had seen better days and started a facility for producing the 57 varieties. We are particularly proud of the fact that we introduced strained baby foods to the Australian market and by doing so, made a significant contribution to the nutrition of the baby population of that country.

Other fellow members of the food processing industry have made similar and significant contributions to the world.

The success of these individual contributions serve only to point up the desperate and ever-increasing need for a more concerted and centralized effort. Therefore, I propose that the food industry explore the possibility and means of making available such knowledge in both food processing and nutritional science as may be useful and applicable to the underdeveloped countries or any areas with critical food problems. This knowledge might or should be made available to our government and through appropriate channels to such agencies as UNICEF, WHO, and FAO.

To repeat, this is how I propose the challenge can be met and how the food industries of the world can assist. To recapitulate—Point one: The creation of scholarships rather than a fund to bring students from all over the world to this country in order to train them in the nutritional sciences, in technology and in related fields. Point two: The creation of grants and endowments to universities to encourage the scope of nutritional study. Point three: The sponsorship of a Nutrition Education Program to disseminate nutritional information to the general public. Point Four: Multi-lingual publication of a journal such as Nutrition Reviews. Point five: The sharing of appropriate food industry knowledge with our government and international agencies working in this field.

In brief, the goal of our industry is the practical contribution toward the betterment of man's nutrition. The task calls for an open mind and a large measure of enlightened thinking.

Mankind has conquered many problems that, at first, seem insurmountable. Through research and increased understanding man is rapidly mastering his own adjustment to all the elements of nature. But in that race, let us not overlook the dignity of man. Man has a right to grow to his full capacity, regardless of who he is or where he was born. The entire civilized world must come to grips with this problem. We who have knowledge must give of that knowledge. We who have substance must give of that substance. The food industry must continue in the fight to banish hunger and malnutrition in any form from the face of this earth.

New activities and goals for the International Union of Nutritional Sciences

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MAY I be allowed to express to your committees and to all those who have helped to make this fifth Congress the overwhelming success that it has been and which has culminated in this magnificent banquet, the thanks and appreciation of the 2,000 people from some 65 nations. I know that these great occasions mean endless preparations—preparations extending over some 4 years. Not only have you followed the general pattern of past congresses but you have added your own individual contributions and your generous measure of hospitality and you have also indicated in no uncertain fashion your concern about the mammoth tasks that lie ahead. It has been said that the peoples who left their native shores of the Old World to make this continent their own were those not given to the art of compromise. Your origins are thus among the individualists and idealists of the Old World, so that mostly everything you do is on an individual and vast scale. You match your heart's desire to give abundantly.

We in the United Kingdom, probably more than any other group from the Old World, tend to compromise—it may at times be a gift in itself—but talking of compromise, I remember a delightful story about an appointment to the headship of one of Cambridge's colleges. The man who was head and shoulders above the other candidates had a dog—a dog from which he would not be parted. But there was a rule in that same college that only cats could be kept by staff, no dogs being allowed. Such, however, was the great gift of compromise that the electors determined to call the dog a cat and so in turn call the candidate of their choice to his high office.

Dr. King, you have invited me to speak about the future of the International Union of Nutritional Sciences. Its aims as you know are

1) To promote international co-operation in the scientific study of nutrition and its applications.

2) To encourage research and the exchange of scientific information in the nutritional sciences, by the holding of international congresses, by publications, and by other suitable means.

3) To set up such commissions or other machinery as may be required in pursuit of above.

4) To provide a means for maintaining contacts with organizations in other fields.

As you know, we have primarily been concerned with making decisions about the location of future congresses. These decisions are made by the Executive Committee which consists of one or two delegates from each country. These delegates should normally be selected by a representative group of nutrition scientists in that country. This is best done by the Nutrition Society or its equivalent of the country and if none exists I would most strongly urge that when you get home you should form one. Such a society should not be exclusively concerned with either human nutrition or with the nutrition of animals of agricultural importance but with all aspects of nutrition. I think that it will have been abundantly clear throughout these meetings, and particularly today, how urgent and necessary it is that such societies be made up of people interested in man and the animals that provide him with his milk, eggs, meat, wool and hide, but also interested in food technology and in public health, welfare and education. Economists and sociologists should also be invited into your group. Such societies or groups having bonded themselves together should nominate national representatives. One such person can then receive official papers about the International Union of Nutritional Sciences (IUNS) and its activities. We welcome Dr. van Eekelen of the Netherlands as our new Secretary General. He has the assistance of Professor Gounelle of Paris.

I think that the IUNS has a role to play as an international body of nutritional workers. And it may be that such agencies of the United Nations—FAO, WHO and the United Children Fund—may be able to make use of us. It might well be that we could help in defining requirements of nutrients. It might also be possible to act as an agency in the setting up of symposia on different problems of an international character. It could act as a clearing house for information on persons working on nutrition in different parts of the world. The IUNS might also help in dealing with questions of international standard definitions and terminology for nutritional conditions and techniques. There are interesting geographic variations in nutritional states which it might help to uncover.

As I mentioned in my remarks on the opening day we are unified by a spirit of urgency about a need which transcends all others. The candles of time are running out. Our business is unfinished. May we be inspired in our attempt to understand the thousand and one secrets of nature yet unravelled and which will provide a more abundant life for us and those who follow after. If so, we shall not have passed these thresholds in vain.

As to our friends of this great continent, the theft of our heart we shall learn to forgive, but you, the thieves, we shall never forget.